HORMONAL CONTROL OF GROWTH OF FRESHWATER AQUATIC PLANTS

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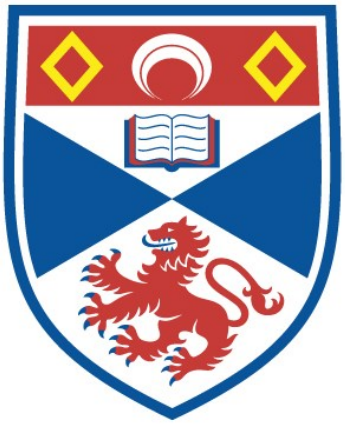
A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1975

A. POTAMOGETON OBTUSIFOLIUS M&K

1. The natural life cycle of this species is described in detail as observed in the Lake of Menteith, near Aberfoyle, Perthshire. For a comparison with results with experimental work, a collection of representative plants from each phase of the life cycle was made.

2. The plant overwinters as turions, which are telescoped plants whichdevelop rapidly from early Spring into the mature elongate plants.

3. The evidence presented in the thesis does not categorically define whether the winter rest period is true dormancy, or whether it is simply an environmentally imposed rest period.

4. At the onset of growth the turions may elongate or exhibit a geotropic growth response. The turions were analysed for gibberellins using the agar diffusate technique and lettuce hypocotyl technique, and gibberellins were tentatively identified as being present in the resting turions. The Avena assay used in the detection of auxin activity in the turions was invalidated due to incorrect experimental technique. The geotropic response of the turions was accelerated when high concentrations of gibberellic acid IAA or sucrose were provided in the external solution, suggesting their involvement. The results of Experiment 6 where only high auxin concentrations of 10^{-4}M accelerated the geotropic response suggests the possible involvement of ethylene. Studies of the soluble carbohydrate changes in the turions at the onset of growth in the loch, and experimentally, using GLC analysis of ethanol extracts demonstrated that there was a dramatic seven-fold increase in the concentration of sucrose over these periods, in the stem of the turions. Since sucrose was shown to

accelerate the geotropic response, then it may be inferred that it is involved in this early stage of growth of the turions. Carbohydrates present in the turions exposed to bathing solutions of IAA, GA3 and sucrose for 24hr. were analysed, and IAA appeared to affect the concentration of sucrose whilst GA3 affected the hexose levels. This evidence and the plasticity of the stem tissue at this stage indicate that auxin may play a significant controlling role.

5. The turions were extracted in hot ethanol and the soluble carbohydrates found using GLC analysis over a temperature range of 100-250°C were fructose , and glucoses, manitol, m-Inositol, and sucrose.

6. High concentrations of meso-inositol were found in the turions and this may explain partially the rapidity of the development of the turions.

7. Since one of the major environmental factors regulating growth is temperature the effect of temperature on the growth of the turions was studied. It was found that all facets of growth stem, leaf, and root growth were partially controlled by temperature. The design of the experiments does not permit a discussion of the relative significance of the role of light in such processes.

8. One of the main aspects of growth studied was stem extension. In nature this occurred most rapidly at temperatures between 15-20°C, during the summer months. Long daylengths and high temperatures are known to increase the turnover of gibberellins, in some terrestrial plants (Zeevart 1971) and thus it was considered worthwhile to study the promotion of growth of the turions by gibberellic acid. A preliminary experiment with hormonal solutions of gibberellic acid, IAA, and sucrose, demonstrated that

gibberellic acid could stimulate the growth of all parts of the turions whilst both IAA, and sucrose stimulated the growth of only certain parts. Further circumstantial evidence in favour of partial control of growth by gibberellins comes from the observation that when 10^{-4}M GA3 was supplied to the turions only then did the plants flower. In nature the plants flower in August when the temperature of the lake is about 20°C, and the daylength is about 14hr.. This flowering of the plants coincides with the period of maximum stem extension. Experiment 12 where the promotion of growth of the turions with gibberellic acid at 5°, 10, 15 and 20°C was studied demonstrated a temperature dependence in the ability to use supplied gibberellic acid. Only when the plants were already active could they utilise it, indicating the importance of other factors in the initiation and the subsequent extension growth.

9. As mentioned in the conclusion to experiment 12, the work brings out two main correlations, growth and temperature, and temperature and the ability of the turions to use supplied gibberellin. The connection between growth and gibberellins, if any, is still therefore tenuous, but in the light of the work at Cambridge (Musgrave et al 1972) it seems reasonably fair to propose the connection as real. Some promotion of growth of the turions was noted in response to supplied ETHREL E.

B. LITTORELLA UNIFLORA (L.) ASCHERS

1. The life cycle of this rosette aquatic plant was discussed in detail.

2. Anatomical differences between terrestrial and submerged forms of the rosettes were found. Terrestrial rosettes were induced very rapidly by exposure of previously submerged

rosettes to air. Lacunal development was greatly reduced in the leaves and roots of the terrestrial rosettes.

3. Two main aspects of the life cycle were considered in detail: propagation of the rosettes; and leaf elongation.

4. Propagation: In nature the rosettes have the ability to propagate by (a) stolon formation or (b) by a less efficient means where the rosette elongates vertically.

(a) Experimental evidence presented indicates that stolon initiation is controlled by gibberellic acid, although cytokinins may be involved. In nature, stolons from similar rosettes may be of varying lengths, and so the elongation phase of stolon development was considered. It was found that gibberellic acid may bring about a protraction of this elongation phase of growth of the stolon, at the expense of the development of the terminal plantlet. Ethylene supplied as ETHREL E inhibited further elongation of the stolons, and furthermore, promoted accumulation of starch. Starch grains in stolons of rosettes treated with gibberellic acid were limited to a central ring of cells. Some radial growth in response to supplied ETHREL E was noted. Stolons may persist in some cases, thus interconnecting many rosettes, but in some cases the stolons senesce. Evidence presented above suggests that gibberellic acid and ethylene may control senescence. Where gibberellin levels are low in the stolons, accumulated ethylene will rapidly effect the senescence of the stolon, thus making the rosette independent.

(b) Where rosettes are partially and repeatedly buried by sediment, they have the ability to perennate themselves vertically by means of an elongated stem axis. Various

rooting levels on the same rosette reflect the sequential nature of the deposition of the sediment. This was found experimentally where rosettes were grown under different conditions of deposition of sediment, and rosettes similar to those experimentally induced were found on a silt fan, near the inflow in Loch Drumore, near Glenshee, in Perthshire. The ability of the plant to propagate so rapidly by stolon formation on eroded shores, or by altering its rooting level and then forming stolons, explains to a great extent the ubiquity of this species throughout Great Britain, and thus implicitly explains the persistence of this species through many of the stages in hydrosere formation.

5. Leaf elongation: Leaf elongation of the rosettes is known to increase with the depth of water in which the rosettes are growing. What is not known is whether this is a function of a changing mud, with an increase in the silt fraction with depth, or whether it is due to the changing light regime, or to both factors.

Experimental evidence presented demonstrates that even in shallow water the leaf elongation of the rosettes may be markedly controlled by the nature of the mud. Thus leaf elongation is partially under nutritional control, and explains the increase in leaf elongation seen in nature, with increasing depth of water. Where there is little change in mud however with depth, the control of leaf elongation must lie elsewhere. The main other variant will be in the light regime.

Factors affected by the light regime will be the levels of hormones such as gibberellic acid, ethylene and auxin. Leaves of the rosettes were able to respond to supplied

ETHREL E when submerged, thus indicating that in shallow water ethylene production may be low in the rosettes. In the saturated system described for Callitriche platycarpa by Musgrave et al (1972) the stems of the rosettes were only able to respond to supplied ETHREL when floating. It is suggested that ethylene may be more actively involved in the radial growth of the leaves, for its stimulation of leaf elongation was not great. Gibberellic acid however, when supplied exogenously stimulated the elongation of leaves initiated during the experimental period. IAA had no effect on leaf elongation, and at levels where induction of ethylene would be expected (10^{-4}M) still no stimulation of elongation occurred. It is suggested that anthocyanin formation in the leaves of the rosettes, lacunal development and hence radial growth are under the control of ethylene, whilst gibberellins have more control over leaf elongation. Such a postulate implies an increase in gibberellins or in turnover of gibberellins with increase in the depth of water, a similar theory to that first forwarded by McComb (1965) but later discounted by Musgrave et al (1972). Both workers were however considering Callitriche species which are either floating or submerged, whilst the rosettes of Littorella uniflora are subjected to degrees of submergence, some of which may affect the light regime significantly to alter hormone levels. Aberg (1943) presented for Lobelia dortmanna, a similar rosette species, field data that indicated that increasing light attenuation brought about increasing elongation of the leaves.

5. High auxin levels in the bathing solutions promoted radial growth of the roots whilst 10^{-4}M gibberellic acid reduced root diameter. Lateral root formation was promoted by high

auxin concentrations and also by gibberellins. There is considerable promotion of lateral roots on aerial exposure of rosettes, and this may be brought about by changes in the levels of these compounds within the rosettes.

HORMONAL CONTROL OF GROWTH OF FRESHWATER AQUATIC PLANTS

The following thesis is presented as original work, for the degree of Doctor of Philosophy, and was carried out over a period of nine terms (1971-4) in the Department of Botany, St. Andrews University, under the supervision of Professor D.H.N. Spence. The work was financed by the Natural Environmental Research Council (N.E.R.C.), in the form of a Research Studentship.

I, Alastair C. Webster, was formally admitted on 6th October, 1971, as a research student under Ordinance General No. 12 and as a candidate for the degree of Doctor of Philosophy.

This is to certify that Mr. A.C. Webster (B.Sc.,B.t.A.) has carried out nine terms of research work under my supervision, according to the conditions in the Resolution and Regulations.

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CHAPTER 1

INTRODUCTION

INTRODUCTION

Compared with the mass of information available for biomass and standing crop estimations for freshwater aquatic plants, there is a distinct lack of basic information concerning the physiology of these plants. Literature to date, concerned with the growth of freshwater aquatic plants is discussed in relevant chapters, and thus does not form the basis of this short introduction.

Questions that remain unanswered on a satisfactor level, with regard to the endogenous and environmental control mechanisms involved are : Why do aquatic plants overwinter in the form that they do?; Are there physiological variations within a species?; Why do most show a sudden spurt of growth in early spring?; What controls the rapid stem growth of some species e.g. members of the Potamogetonaceae; what controls root production, and what are the functions of these roots?; What controls the shunting of necessary metabolites into certain growth processes at the expense of others e.g. stolon production?; and What controls when the plants will senesce?

A pre-requisite for sensible management of freshwater reserves is a close working knowledge of the life cycles of the endemic species of aquatic plants, with regard to environmental and endogenous factors inducing phase changes in the plants. A knowledge of when a particular species was in the turion phase of its life cycle, for example, would allow removal or 'seeding' of the species purely by predictable mechanical means, that is would avoid the

need for chemical control.

Aquatic plants are ideal tissue for hormonal studies of growth control. In the species studied below, the whole 'gamut' of plant development from a telescoped winter phase is open to investigation - overwintering, termination of the rest period, geotropism, stem extension, root emergence and development, leaf elongation, inoonal development, flowering, formation of the resting winter phase on the mature plant, abscission of these turions, and finally senescence of the parent plant. It is not often in terrestrial species that such a comprehensive array of processes is so readily open to investigation.

The aim of the following thesis is to study phases in the life cycles of two aquatic plants: Potamogeton obtusifolius Mert & Koch and Littorella uniflora L. Aschers. Potamogeton obtusifolius M&K is a submerged species, whilst L. uniflora L. Aschers. is a rosette species which may grow terrestrially, emergent or submerged. The thesis concentrates on the nature of the rest period, and stem extension in P. obtusifolius M&K, and on the leaf development and propagation of L. uniflora L. Aschers. An attempt is made throughout to compare the findings of the experiments with the recorded and observed development of the plants under natural conditions.

Experimental procedures

In many of the experiments plants were held in bathing solutions of hormones for 18hr. or 24hr. periods. Local application of hormones to aquatic plants is not practicable, and hence the use of solutions. When held in these solutions, the plants were generally kept in the dark, to avoid complications with photolysis effects. Where the plants were held in the solutions in the light, then the interpretation of the results must be more tentative. When the plants were held in the hormone solutions for the duration of the experimental period, then there is the possibility of bacterial degradation of some of the hormone before penetration of the tissue. Repeated extractions over a growing season, of the endogenous hormones and inhibitors present in the turions, would have been carried out had time permitted. Similarly once a suitable system had been found levels of evolved ethylene would have been monitored.

Experimental design and interpretation is limited in some cases by the lack of required basic data, such as meaningful light data. Light is an extremely variable environmental factor diurnally and seasonally, and as no device was available for continuous spectrophotometric recording of the natural light regime, no light data is furnished. Cultural facilities were rather inadequate, thus also limiting experimental design.

CHAPTER 2

LIFE CYCLE OF POTAMOGETON OBTUSIFOLIUS M&K

CHAPTER 2 CONTENTS PAGE

PLATE 1. Collection site at the Lake of Menteith.

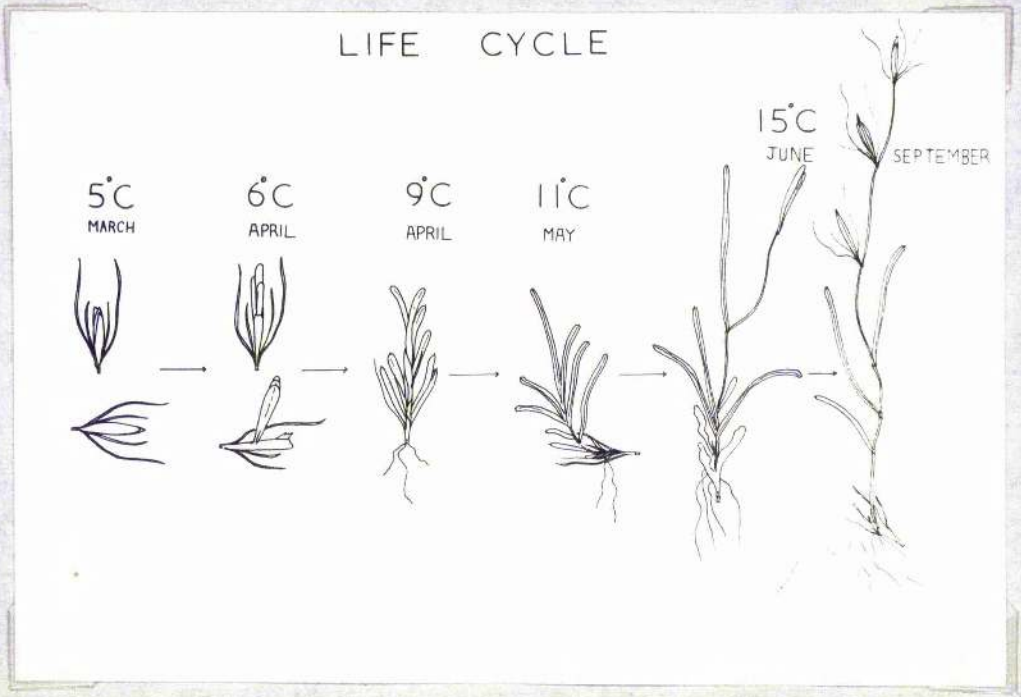


FIG. 1. Diagrammatic representation of life cycle of Potamogeton obtusifolius M&K. (not to scale)

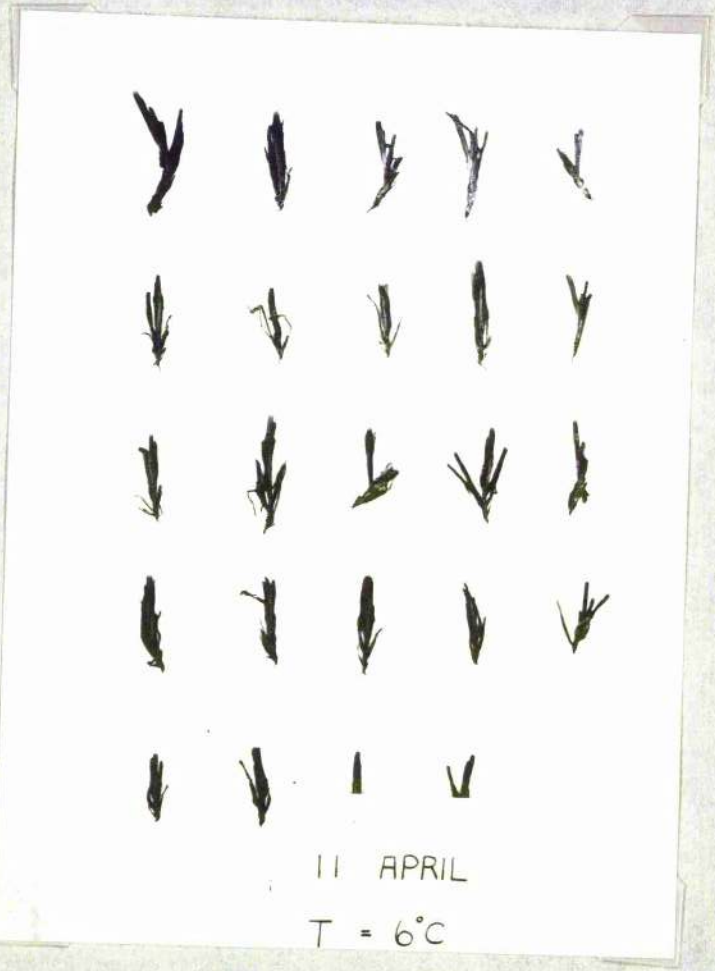


PLATE 2. Turions collected from Lake.
11th APRIL

PLATE 3. Turions collected from Lake
28th APRIL

PLATE 4. Turions collected from Lake.
18th MAY

PLATE 5. Turions collected from Lake.
28th JUNE

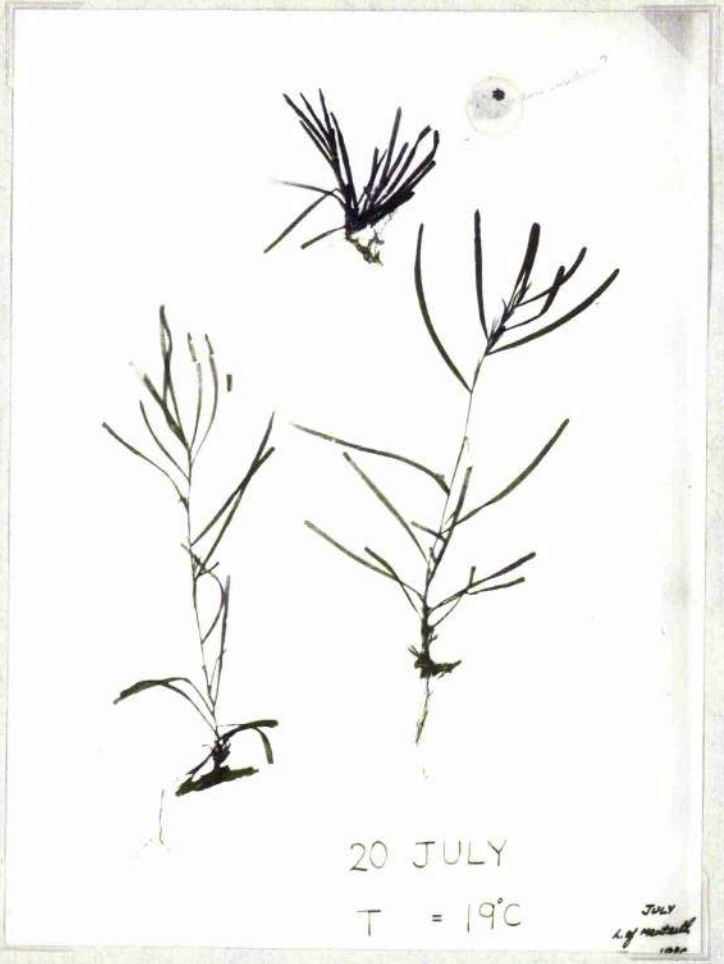


PLATE 6. Turions collected from Lake.
20th JULY

PLATE 7. Turions collected from Lake.
2nd SEPTEMBER

THE LIFE CYCLE OF P. OBTUSIFOLIUS MEM

The life cycle is illustrated in plates 2-7, and is based on a collection of material during different phases of the life cycle of the plants, from the Lake of Menteith (see plate 1). Figure 1 summarises the life cycle in diagrammatic form. The turions, which are described in full in page 14 are the specialised winter buds of the species. Despite the lack of a root system, which is reported to be required for the maintenance of healthy leaf tissue, through the supply of cytokinins, the leaves are a dense green colour. By mid-April (see plate 2) visible growth occurs with stem extension occurring either parallel to the long axis of the turion, or at right angles to it, depending on the orientation of the turion. Such initial stem extension however is very slight, and stem extension is shown later to be temperature dependent. By the end of April (see plate 3) the temperature of the loch has risen to 9°C, and all the turions have now begun growth, with marked stem elongation, and the central bud of leaves is opening. The pre-formed root primordia are beginning to emerge from the basal parts of the turions, at each node. By mid-May (see plate 4) the turions have been transformed into recognisable plants, with an elongating stem, leaves that are separate and elongating, and root development is now well underway. Throughout June, July and August there is marked stem extension, and the leaves also elongate. A great mass of root tissue is never formed. By September

the plants are flowering (see plate 7) apically, and every side shoot is now a turion i.e. there is no further stem growth. Whilst on the plant the turions develop into the squat overwintering buds, and these are abscissed, when the parent plant senesces, falling in masses to the mud surface, there to overwinter. Deep-water forms, with more internodes are more efficient propagating units, for they both flower and form turions. Shallow water forms (see plates 6,7) found did have turions but the plants were rosette-like (see asterisked cases in plates 6,7). This photo-inhibition of stem growth is supported experimentally.

The overwintering phase in the life cycle is now considered in some detail.

OVERWINTERING

Aquatic plants overwinter in a variety of ways:

1. Potamogeton pectinatus contracts into short tubers rich in starch reserves.
2. Potamogeton praelongus may die back to the extensive rhizome system.
3. Elodea canadensis & Myriophyllum alterniflorum overwinter as dormant shoot apices attached to the parent plant.
4. Potamogeton crispus may overwinter as the intact plant, with a reduced metabolic rate or it may form overwintering leaf structures (Arber 1920).
5. As turions which are axial structures, e.g. Potamogeton obtusifolius M&K. These structures are basically telescoped plants.

The mode of overwintering may be modified by the nature of the winter season and so the above listed modes are not the only modes of overwintering for each species mentioned. Sculthorpe (1967) deals with overwintering structures in detail.

POTAMOGETON OBTUSIFOLIUS M&K

This species of submerged pondweed overwinters as specialised winter buds called turions. (see plate 2). The turions have a central bud of about 8 leaves at the time of formation of the turions. Ligule-like scales separate the leaves in this bud. The stem tissue is about 2cm. in length and consists of telescoped internodes, and has some development of lacunae. The basal part of the turion

consists of closely packed parenchymatous tissue which is densely packed with discrete starch grains. (see plate 8). The grains stain with iodine, are anisotropic (indicating accretion of material in layers) and addition of dilute alkali to suspensions of the grains caused the visible separation of the 2 phases, amylase and amylopectin. The plastids in the cells near the epidermis have starch staining bodies in them. (see plate 9). Root primordia are present in the turions at the time of their formation (see plate 10). This germ plant is thus geared anatomically for an instant response to more favourable conditions for growth. Despite the lack of a functional root system for the supply of cytokinins, the leaves of the turion are a dense green colour.

The turions overwinter on the mud, and in the mud, in all orientations, from mid-October until mid-April. Since no visible morphological change occurs in the turions over this period, whatever is occurring metabolically is not effecting growth.

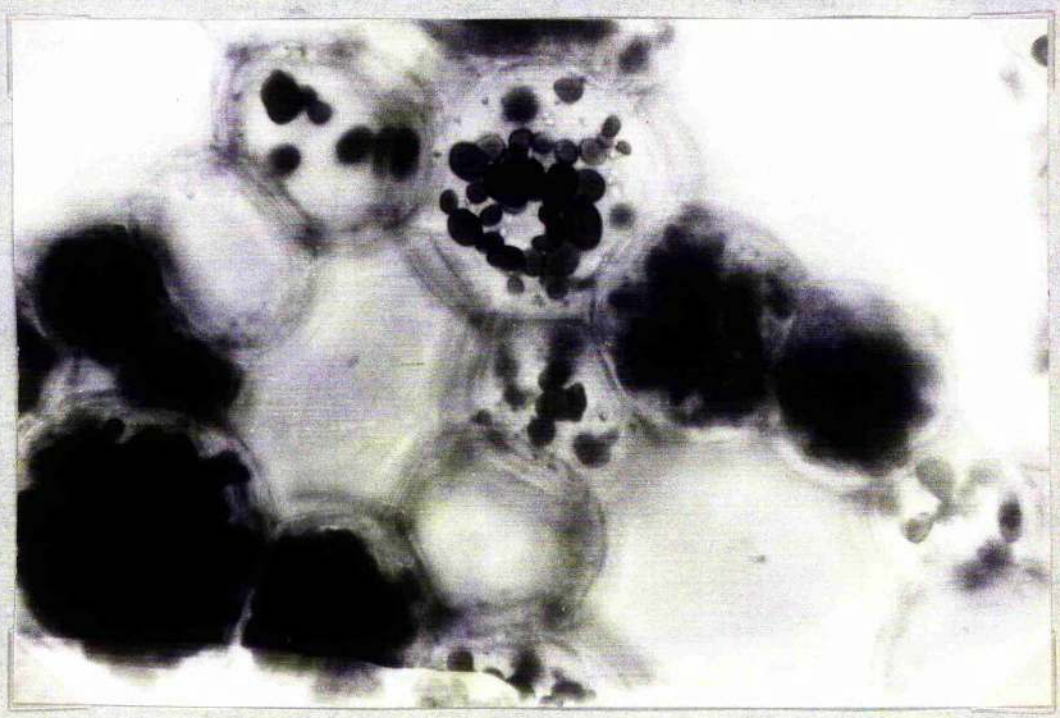


PLATE 8. Starch grains in parenchyma cells of turions.

PLATE 9. Starch formation in the plastids.

PLATE 10. T.Section of turion stem, showing root primordia present in March.

Having considered this overwintering phase, stem, leaf and root growth is now studied.

Examples from the natural collection, illustrated in plates 2-7, were scored for various growth parameters, and these values were plotted against the temperature of the lake (see figure 2), thus allowing a comparison of the results from the laboratory experiment, which follows, with those obtained from examples from the natural collection.

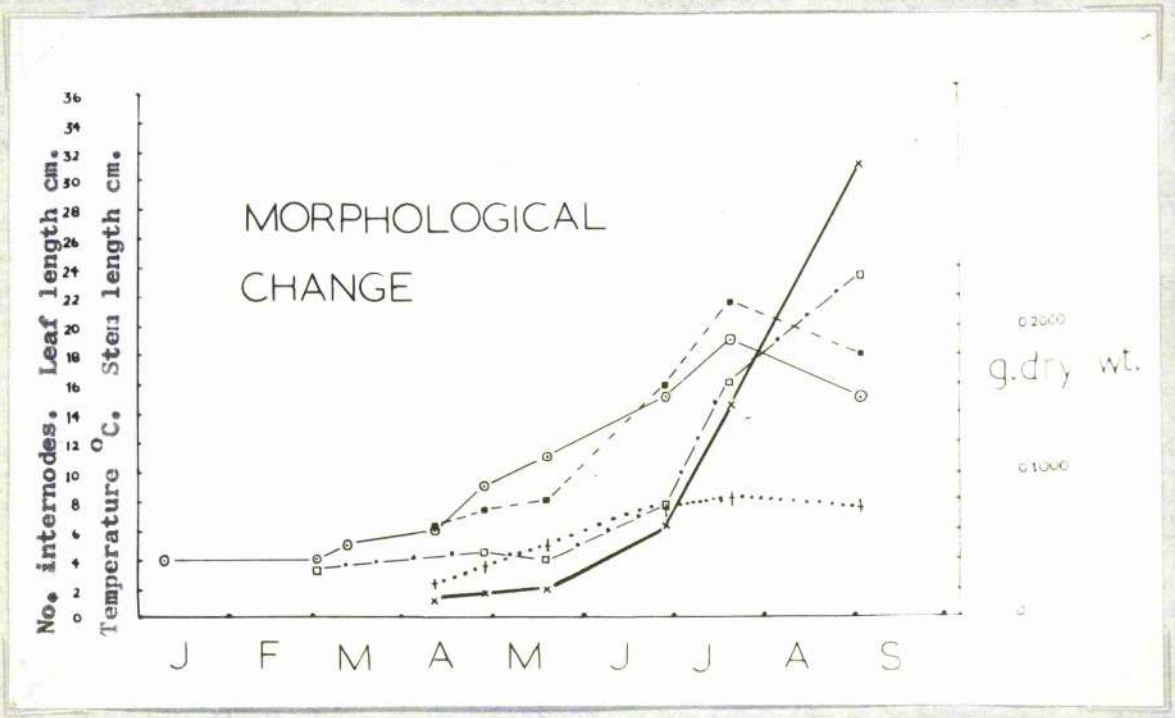


FIG. 2 Growth of plant parts throughout the season, in the Lake of Menteith in relation to the temperature of the lake.

Legend: Temperature ⊙—⊙—⊙

Stem length ×—×—×

Internodes ■- - -■- - -■

Dry weight □—·—□·—·□

Longest leaf †·····†·····†

The significances of the differences between the means, and the standard errors are presented in table 1, page 19 (estimated using the Students 't' test)

DATE	TEMP. °C	STEM LENGTH (cm.)	INT.NO.	DRY WEIGHT (mg.)	LONGEST LEAF (cm.)
JAN. 9	4	-	-	-	-
MARCH 2	4	-	-	33.8±6(12)	-
MARCH 12	5				
APRIL 11	6	1.25±0.09	6.17±0.37(6)	-	2.4±0.05(6)
		P 0.01	NS		P 0.001
APRIL 28	9	1.75±0.12	7.33±0.45(6)	46.4±2.6(12)	3.6±0.01(6)
		NS	NS		P 0.001
MAY 18	11	1.90±0.04	8.00±0.24(6)	40.4±3.4(11)	5.0±0.20(6)
		P 0.01	P 0.001		P 0.001
JUNE 28	15	6.32±1.36	15.83±1.19(6)	76.6±4.7(12)	7.4±0.30(5)
		P 0.01	NS		NS
JULY 20	19	14.5±0.71	21.50±1.06(2)	1600±11.2(12)	8.2±0.8(4)
		NS	NS		NS
SEPT. 2	15	31.12±11.53	18.0±1.58(4)	233.6±3.8(9)	7.5±0(2)

Figures in brackets indicate the number of plants scored for listed parameters on each occasion. Plants harvested were dissected into the various parts.

TABLE 1 Significance of differences in means for various growth parameters of plants from the Lake of Menteith, during one growing season. (Student's 't' test used in the estimation of the significance of the differences between the means).

Having collected specimen plants from each phase of the life cycle, turions were grown at four different temperatures, with a common light regime, to determine what control temperature exerted over the development of the turions. This was done in the following experiment.

EXPERIMENT 1

AIM: To investigate the effect of temperature on the development of the turions of P. obtusifolius M&K.

MATERIALS: Turions were collected from the Lake of Menteith, and were selected for the experiment if they measured 3.5-4.0cm. from the base of the stem to the apex of the leaves.

METHOD: The growth containers in this experiment were 1 litre beakers as shown in plates 11-14, which contained a constant amount of John Innes Potting Compost No. 1, and were topped up with distilled water. The turions were planted horizontally, for if planted vertically grew towards the sides of the containers. Three turions were planted in each beaker. The turions were grown at 5, 10, 15 and 20°C. The beakers were held in constant temperature baths, which had coupled Tecam dip coolers and heaters. Illumination was provided for 14 hr. per day by 3 40 watt gro-lux tubes, which were 4 feet in length, and were 40cm. above the tops of the beakers. There were three sampling occasions, 28th May, 4th June, and 11th June, and nn these occasions the plants were dissected into leaf, stem and root, and scored for various parameters. Sample turions were not measured at the start of the experiment because of shortage of material. Since no growth occurr over the experimental period at 5°C, the initial means for each paramater at 5°C are taken as defining the morphology of all the turions at the start of the experiment. These values are represented by the shaded histograms in figures 3-7. The sampling occassions are labelled 1-4 in figures 3-7.

RESULTS: Growing turions are illustrated under the experimental conditions 11 days after planting, in plates 11-14. Figures 3-7 illustrate the results obtained for the development of the various parts of the turions at the different temperatures. Initial and

final means for each growth parameter were compared to determine if, for example any stem growth had occurred at 10°C over the experimental period, and the statistical significances of the differences of such means (from the histograms) are presented in table 2. Table 3 presents the final means for each parameter at the four temperatures and states whether there are any statistical significance to such differences.

TEMPERATURE	STEM LENGTH	NITS.	LEAF LENGTH	LEAF NO.	ROOT LENGTH
5°C	NS	NS	P<0.05	NS	NS
10°C	NS	NS	NS	NS	P<0.001
15°C	NS	P<0.02	NS	P<0.001	P<0.001
20°C	P<0.05	P<0.001	P<0.001	P<0.001	P<0.001

TABLE 2. Significance of differences between initial (17th May) and final (11th June) means for various growth parameters at 5, 10, 15 and 20°C.

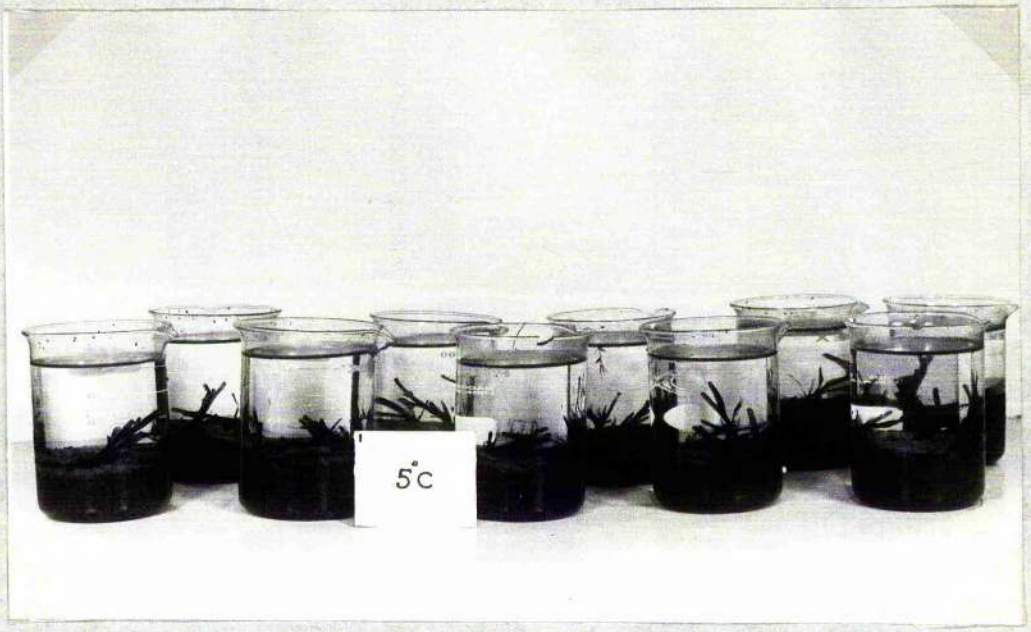


PLATE 11. Growth of turions at 5°C.

Three gro-lux tubes provided illumination for 14hr. per day at the four temperatures used in the experiment.

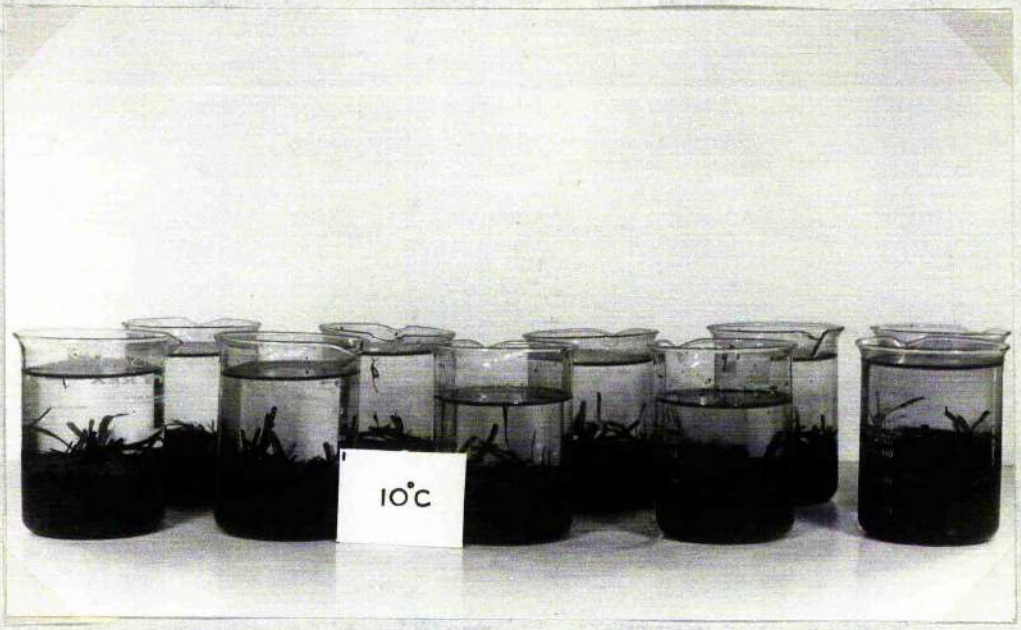


PLATE 12. Growth of turions at 10°C.

PLATE 13. Growth of the turions at 15°C.

PLATE 14. Growth of the turions at 20°C.

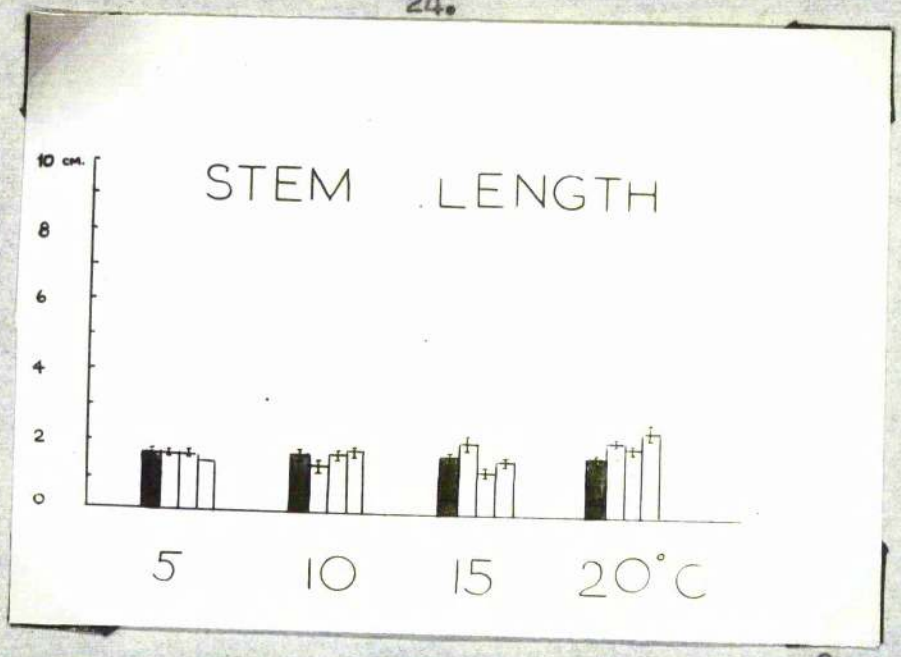


FIG. 3. Stem development at 5,10,15 and 20°C.
Shaded histograms in figures 3-7 define the morphology of the turions at the start.
Results presented as MEAN ± SE.
1 11th May
2 28th May
3 4th June
4 11th June.

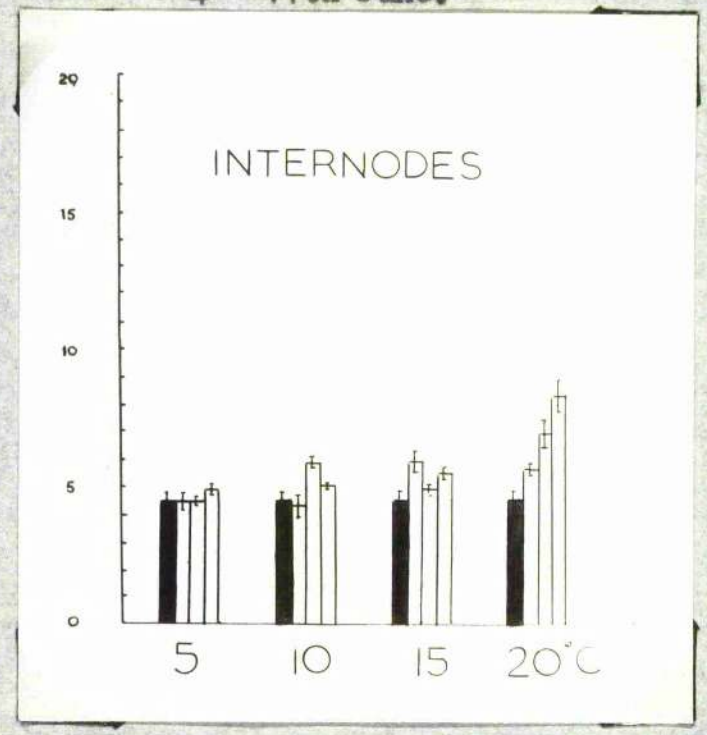


FIG. 4. Internode production at 5,10,15 and 20°C.
Results presented as MEAN ± SE.

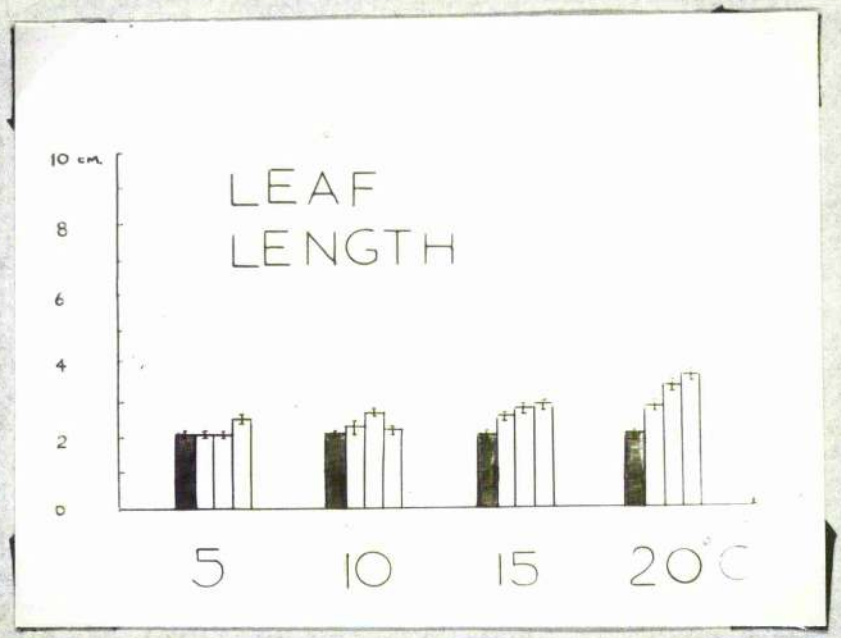


FIG. 5 Leaf elongation at 5,10,15 and 20°C.
Results presented as MEANS ± SE.

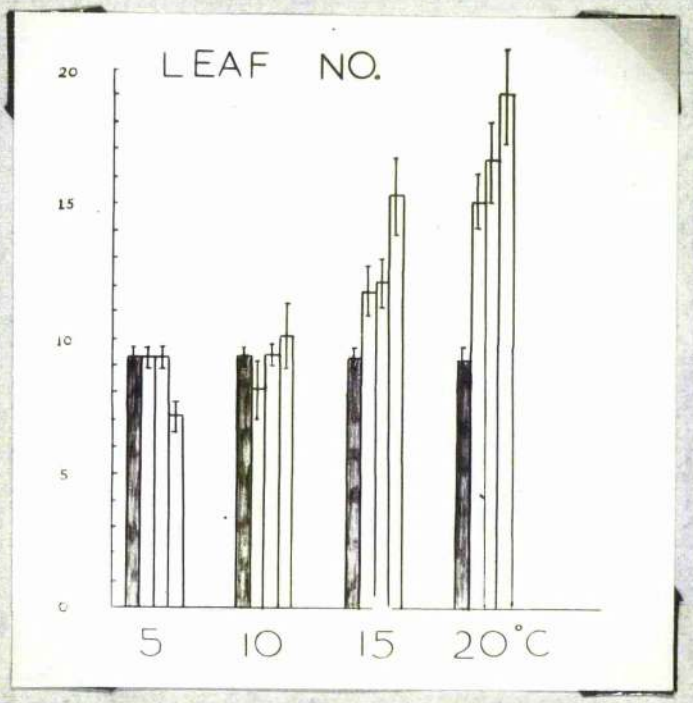


FIG. 6. Leaf production at 5,10,15 and 20°C.
Results presented as MEANS ± SE.

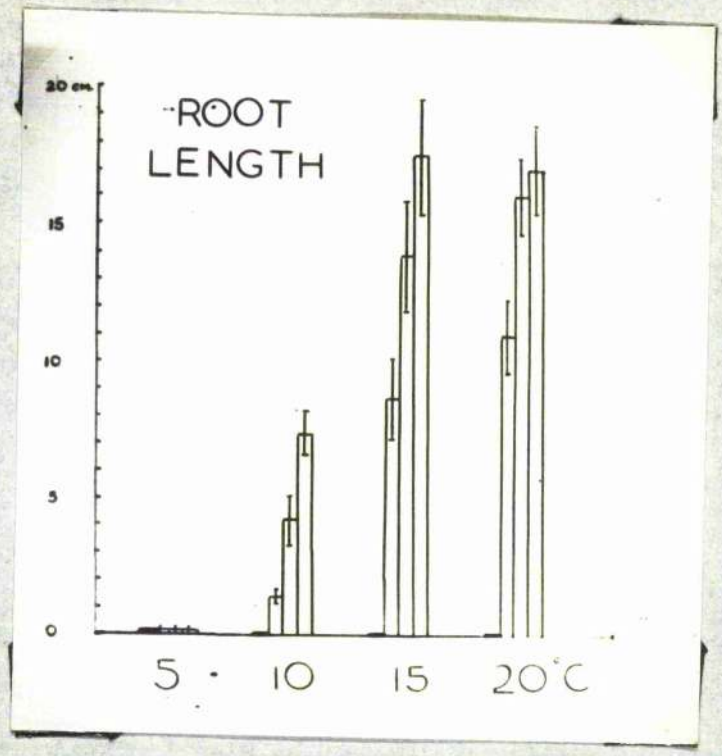


FIG. 7. Root growth at 5,10,15 and 20°C. Results presented as MEAN ± SE.

TEMPERATURE	STEM LENGTH	INTS.	LEAF LENGTH	LEAF NO.	ROOT LENGT
5°C	1.5±0	4.9±0.2	2.5±0.17	7.1±0.6	0.2±0.
	(P 0.01)	(NS)	(NS)	(NS)	(P 0.0
10°C	1.8±0.09	5.0±0.2	2.2±0.1	10.1±1.2	7.3±0.
	(NS)	(NS)	(P 0.01)	(P 0.01)	(P 0.0
15°C	1.6+_0.1	5.5±0.2	2.9±0.17	15.3±1.4	17.5±2.
	(P 0.01)	(P 0.001)	(P 0.01)	(NS)	(NS)
20°C	2.5±0.2	8.3±0.6	3.8±0.17	19±1.8	17.0±1.

TABLE 3. Final means for growth parameters at 5,10,15 and 20°C with significance of means differences for parameters from plants from different temperature treatments (5°C/10°C; 10°C/15°C; 15°C/20°C).

DISCUSSION:

Internode production: Fig. 4 indicates that at 5°C and 10°C there was no production of new internodes, but at 15°C there was, and more so at 20°C (final means with significance levels of means differences presented in table 3).

In the loch the number of internodes does not increase significantly until the temperature of the loch is above 10°C (see fig. 2). Even under lower light intensities therefore internode production is temperature limited.

Stem extension: Significant stem extension of the turions only occurred at 20°C.

Since significant stem extension takes place in nature (see fig. 2) at 6-8°C over a shorter period of time than the duration of this experiment the light regime of the experiment must be considered to inhibit stem extension. This is later shown to be the case, in experiment 2 where an inverse relationship is found between daylength and stem extension. Under the experimental conditions at 20°C therefore a twofold temperature effect operated:

a. Removal of the experimentally imposed light inhibition of growth.

b. Temperature stimulation of extension (as in nature).

Perhaps at higher temperature there is increased production/turnover of gibberellins which may allow growth even in the presence of any light-induced inhibitors. This possibility is discussed further in chapter 4.

Leaf elongation: There was no significant leaf elongation

at 5°C or 10°C but at 15°C there was, and even greater elongation at 20°C (see table 3 for final means). Likewise the temperature threshold for leaf elongation was much higher than that found in nature (see fig. 2), thus again suggesting an inhibitory effect of the light regime.

Leaf production: (See fig. 6) Leaf production at 5°C or at 10°C was not significant, but there was considerable increase in leaf number at 15°C and 20°C, although the final means were not significantly different. No figures are available for seasonal change in the number of leaves per plant. Using sugar beet and mangold, Watson and Baptiste (1938) were the first workers to correlate rate of leaf production and temperature. They also noted that when the temperature rose after a sharp fall, leaves were produced much more rapidly than would be expected from the prevailing temperature which I suggest may be a temperature stimulation of gibberellin synthesis after a cold period followed by the transfer to a higher temperature.

Root growth: No root primordia emerged from the turions held at 5°C. At 10°C, and above, the root primordia did emerge, and developed more rapidly with increasing temperature (final means for 15°C and 20°C are not significantly different (see table 3 and fig. 7).

The root primordia in turions in the loch did not emerge until the temperature of the loch was 8-10°C (see plate 3). The development of the root primordia is later shown to be under the control of hormonal and nutritional factors (carbohydrates) whose synthesis and metabolism are temperature dependent. With reference to root primordia (lateral roots)

in forest trees, Shapiro (1958) reported that the formation growth of the root primordia may be inhibited by light, especially if the light reaches the area of tissue where they are developing, or normally would develop. There appears to have been little of such an effect here as root growth was initiated at a similar temperature to that found in nature. That only roots developed over the experimental period at 10°C may be a consequence of the greater sensitivity to low hormone levels of roots compared with shoots. Hormonal agents and concentrations causing the emergence of the root primordia are not necessarily the same as those causing development. Furthermore, geotropic stimulation may have brought about a local concentration of endogenous hormones in the vicinity of the root primordia.

SUMMARY:

1. A laboratory comparison was made at 5, 10, 15 and 20°C, with the growth of the turions under natural conditions in the Lake of Menteith.
2. High temperature thresholds compared with those found in nature for growth of various parts of the turions indicate that the experimental light regime was inhibitory to stem and leaf development.
3. The above findings indicate a light and temperature interaction. To differentiate between temperature stimulation of growth and light effects, a range of light intensities could be used at the 4 temperatures, and the 'independent' effects of light and temperature ascertained

using analysis of variance.

4. The observed low temperature threshold for root emergence and development, when no other parts are developing, may suggest a certain hormonal independence of the roots.

Having postulated that the experimental light regime of 14hr. illumination was inhibitory to the stem elongation of the turions, the following experiment is designed to investigate the effect of different daylengths on the stem growth of the turions.

EXPERIMENT 2

AIM: To investigate the effect of daylength on the stem growth of the turions of P. obtusifolius M&K.

MATERIALS: Turions were selected for the experiment if they were 4cm. in length from the stem base to the leaf apices. Plants were grown in 1600ml. John Innes Potting Compost No. 1, and were held under 16cm. water.

METHOD: Four plants were grown at each daylength of 2,4,8 and 14hr. Illumination was provided by 3 40 watt gro-lux tubes, which were 4 feet in length, and held 30cm. above the surface of the water in the plastic bins. All plants were grown at 20°C. The plants were grown for 3 weeks (3-28th April, 1974), when there were obvious differences between plants from the different treatments.

RESULTS: Results are presented below, in tables 4 and 5 as MEANS + S.E., and the plants are illustrated in plate 15.

DAYLENGTH	STEM LENGTH (cm.)	NO. INTERNODES
2hr. (4)*	4.67±0.33	13.75±0.89
4hr. (3)	3.52±0.12	12.3±1.09
8hr. (4)	3.11±0.52	11.75±1.63
14hr. (4)	2.57±0.16	11.0±0.50

TABLE 4 Relationship between the stem growth of the turions of P.obtusifolius M&K and the period of illumination, when the plants are grown at 20°C.

* The figures in brackets indicate the number of replicates.

	STEM LENGTH	NO. INTERNODES.
2&4	P 0.02	NS
2&8	P 0.02	NS
2&14	P 0.01	P 0.05
4&8	NS	NS
4&14	P 0.01	NS
8&14	NS	NS

TABLE 5 Significance levels for differences between means from Table 4.

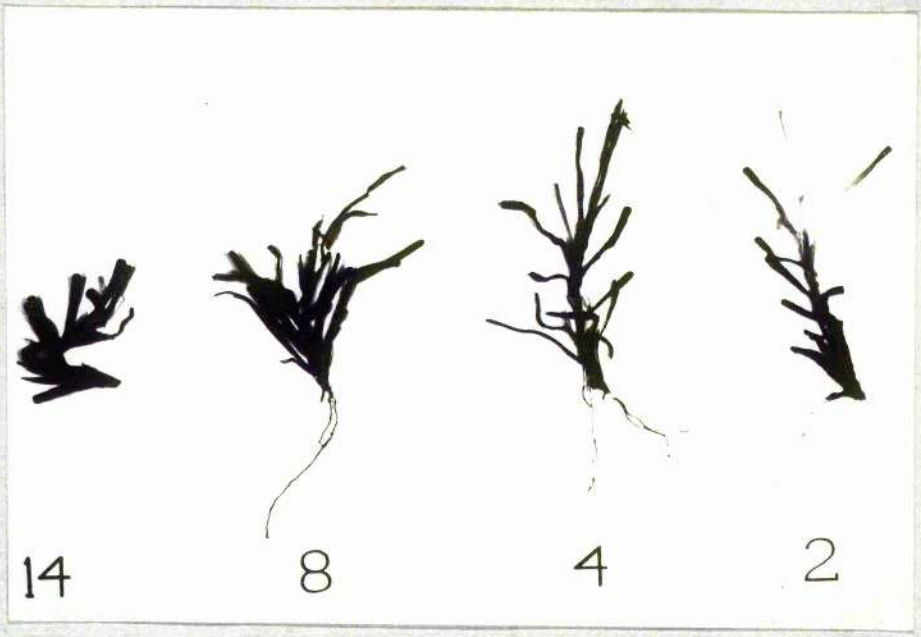


PLATE 15. Growth of turions in compost, under 16cm. water at 20°C at daylengths of 2,4,8 and 14hr. (three gro-lux tubes)

DISCUSSION: Thus, experimentally, an inverse relationship exists between the duration of the period of illumination and the stem development of the turions. There was little effect on internode production, although the stems were not examined microscopically. With 14hr. illumination the stems had hardly developed from the turion stage (see plate 15) and thus the light is inhibiting growth of the stems. This inhibition of stem extension may explain the rosette nature of several plants found in four foot depth of water in the Lake of Menteith (see plate 6). The gro-lux tubes have a large peak in the red region of the spectrum, and thus the inhibition of growth may be red-light inhibition.

The natural light regime would be dimmer than that of the cultural conditions used in any of the experiments. Turions were found elongating under the mud debris where the light intensity would be very low. Rapid stem elongation under such conditions was confirmed experimentally (see figure 8b, page 54), where turions were buried and stem elongation was very rapid.

CHAPTER 3

TERMINATION OF THE REST PERIOD OF THE TURIONS

CHAPTER 3 CONTENTS PAGE

EXPERIMENTS :

Isolation of hormones in aquatic plants

Introduction

Few experiments have been carried out to isolate growth substances from aquatic plants. Homes & Schoor (1937) demonstrated apical dominance in Elodea canadensis using an agar block technique. Similar studies were carried out more recently by Fleury (1966) who found that with Marsilea drummondii, apical dominance was controlled by hormonal and nutritional factors. Pieterse et al (1971) isolated gibberellin fractions from Wolfiella floridiana, and found qualitative and quantitative differences in gibberellins in the rest phase, which are turions, and in the actively growing free-floating phase. In 1972 Musgrave, Jackson and Ling, found that when the stems of Callitriche platycarpa were submerged, ethylene was produced, and accumulated internally, promoting elongation. Similarly, Musgrave and Walters (1973) demonstrated ethylene production in petioles of Ranunculus sceleratus. Qualitative and quantitative work on endogenous hormones in aquatic plants is thus seen to be scanty.

Experiments 3 and 4 attempt to demonstrate the presence of gibberellin and auxin activity in the turions of Potamogeton obtusifolius M&K.

EXPERIMENT 3

AIM: To determine if gibberellins are present in the resting turions of Potamogeton obtusifolius M&K.

MATERIALS: Ionagar no.2 Oxoid Ltd. The lettuce seed used was Cabbage lettuce seed (Fortune) Suttons. Turions were collected from the Lake of Menteith on March 2nd, 1974.

METHODS: The agar diffusate technique first employed by Jones and Phillips (1964) was used. One half of a turion was placed on 15ml. of sterile 1% Ionagar, using 12 test plates and 4 controls. After 14hr. the plant parts were removed, and 1ml. of distilled water was added to each plate, and then 5 lettuce seeds were placed on each plate. The plates were held at 20°C in daylight. Six days later the hypocotyls were measured. Measuring was carried out by placing the seedlings on graph paper under a glass slide to keep the hypocotyls straight. No purification of the agar diffusates was carried out, and the gibberellin activity was not determined quantitatively from a dose-response curve. The lettuce hypocotyl bioassay for gibberellins was proposed by Frankland and Wareing (1962). Whether it was legitimate to halve the turions lengthwise using a diffusion technique is not known. Ideally a much smaller volume of agar should have been used.

RESULTS: The results are presented below in table 6.

PLATE *	LETTUCE HYPOCOTYL (cm.)	MEAN	SD.
1	1.1,0.8,0.8,0.8,0.5	0.80	0.19
2	1.0,0.8,0.6,0.9,0.5	0.76	0.19
3	0,0,0.5,0.5,0.5	0.30	0.24
4	0.7,0.8,0.5,0.7,0.5	0.64	0.12
5	0.4,0.3,0.3,0.5,0.3	0.36	0.08
6	0,0,0.7,0.7,0.2	0.32	0.31
7	1.0,1.0,1.1,1.0,0.8	0.98	0.10
8	0.5,0.5,0.5,0.5,0.5	0.50	0
9	0.2,0.5,0.5,0.5,0.4	0.42	0.12
10	0,0.5,0.8,0.8,0.7	0.56	0.30
11	0.4,0.3,0.4,0.3,0.2	0.32	0.07
12	0.4,0.5,0.7,0.5,0.3	0.48	0.13
C1	0.8,0.5,0.5,0.5,0.5	0.56	0.12
C2	0.3,0.3,0.3,0.2,0.2	0.26	0.05
C3	0.4,0.4,0.5,0.2,0.2	0.34	0.12
C4	0.3,0.4,0.3,0.2,0.2	0.28	0.07

			SD	
GRAND MEANS	CONTROL	0.36	0.28	(20)
	TEST	0.54	0.15	(60)

* PLATES 1-12, C, -C4 refer to Petri dishes each containing 5 seeds.

TABLE 6 Lettuce hypocotyl bioassay for gibberellins - lengths of lettuce hypocotyls from control(C) and test plates. (see page 37 for details)

The student's 't' test indicates thatthe significance of the difference of the two grand means is $P < 0.001$. The individual means from each plate were then analysed by the Mann-Witney ranking method, for unequal samples.

MEANS	CLASS	RANK
0.26	n1	1
0.28	n2	2
0.30	n1	3
0.32	n2	4.5
0.32	n2	4.5
0.34	n1	6.0
0.36	n2	7
0.42	n2	8
0.48	n2	9
0.50	n2	10
0.56	n1	11.5
0.56	n2	11.5
0.64	n2	13
0.76	n2	14
0.80	n2	15
0.98	n2	16

TABLE 7 Mann-Witney test applied to results presented in table 6. Legend: n1 refers to control
n2 refers to test plates.

The Mann-Witney test does not assume normal distribution of the samples. In the above table, summing the n1, we obtain $T_1 = 21.5$ and the table values for T at P 0.01=21, and at P 0.05 = 26, and thus the controls differ significantly from the test plates, implying gibberellin activity in the turione. Diffusion occurred at $20^{\circ}C$, and thus some of the activity detected may have resulted from temperature stimulation. Furthermore, wound gibberellins have been

reported (Rappaport and Sachs 1967).

The above statistical analyses demonstrate that the turions contain gibberellins.

DISCUSSION: The only reported isolation of gibberellins in aquatic plants has been carried out by Pieterse et al (1971) from a member of the Lemnaceae, Wolffiella floridiana. The system they described, probably operates in the life cycle of P.obtusifolius, which also overwinters as turions, and where the development of the plant is characterised by the rapid elongation of telescoped internodes in the late Spring.

EXPERIMENT 4

AIM: To determine if auxins are present in the turions of P. obtusifolius M&K.

MATERIALS: The agar used was Ionagar no. 2, and the Avena seeds used were

METHOD: The Avena curvature test was used in this experiment to detect the presence of auxin material in the turions.

Turions were not sterile, but were washed thoroughly in distilled water to remove adhering algae. They were then halved lengthwise. 3ml. sterile Ionagar no. 2 was added to each Petri dish, and four halves of turions were placed on the agar in each dish for 24hr. Twelve sample Petri dishes were used and 5 control plates. The experiment was carried out in the dark under a green safe light at 20°C. Coleoptiles 1cm. in length were chosen, decapitated once. After the diffusion period of 24 hr. $2mm^3$ agar blocks were cut randomly from each Petri dish, and these blocks were applied unilaterally to the prepared coleoptiles. Surface creep of auxin was prevented by the careful insertion of coverslip pieces into the tips of the coleoptiles. The angles that the coleoptiles made with the vertical were measured 21 hr. later. The duration of this experimentwas 6-7th April 1974.

This experiment was carried out before it was known by myself that by a 'regeneration of the physiological tip' the coleoptiles regain the ability to produce IAA within 3 hr, after a first decapitation. The results presented below have thus little meaning, and the experiment must therefore be seen largely as an exploratory exercise.

RESULTS: The results obtained are nevertheless presented to illustrate the statistical analysis of the results that would be carried out were the experiment repeated properly.

SAMPLE NO*	ANGLE	MEAN	SD	SE
1	45,25,24,25,32,31	30.3	7.2	3.0
2	11,0,35,30,0,0	12.7	14.6	6.5
3	20,14,0,0	8.5	8.8	4.4
4	20,26,0,20,15,20	16.8	8.2	3.3
5	25,12,0,15	13.0	8.9	4.5
6	22,20,0,0	10.5	10.5	5.3
7	20,10,12,0	10.5	7.1	3.6
8	20,0,18,0	9.5	9.5	4.8
9	20,20,34,20,0,15	18.2	10.0	4.1
10	32,30,11,25	24.5	8.2	3.1
11	21,15,0,0,14,16	11.0	8.1	5.3
12	15,30,0,15,0	12.0	11.2	5.0
C1	0,0,12,0,0,	2.4	4.8	2.1
C2	0,0,0,10,12	4.4	5.4	2.4
C3	0,0,0,0	0	0	0
C4	0,15,20,0	8.8	8.9	4.5
C5	0,0,15,15	7.5	7.5	3.7

Grand means: Control = 4.50 S.Deviation = 6.8 No.

No. coleoptiles = 58 Diffusates = 15.52

S.Deviations = 12.93 No. coleoptiles = 22

* Sample numbers refer to individual Petri dishes, from which agar cubes were cut.

TABLE 8 Avena curvature test for diffusates from turions of P. obtusifolius M&K

The student's 't' test applied to the above means indicates a significance in the difference of the means of $P<0.001$. The means for each plate were also analysed using the Mann-Witney ranking technique for unequal samples.

Avena coleoptile - mean angle of curvature	Class	Rank
0	n1	1
2.4	n1	2
4.4	n1	3
7.5	n1	4
8.5	n2	5
8.8	n1	6
9.5	n2	7
10.5	n2	8.5
10.5	n2	8.5
11.0	n2	10.0
12.0	n2	11.0
12.7	n2	12
13.0	n2	13
16.8	n2	14
18.2	n2	15
24.5	n2	16
30.3	n2	17

TABLE 9 Avena curvature test on diffusates from turions. Mann-Whitney test on means for angle of curvature. n1 refers to the controls, n2 refers to the test plates.

Using the formula for T, $T_1 = 16$, and the table value for T at P 0.01 is 21, and so there is a highly significant difference between the control and test plates i.e. the turions contain auxins. Had chromatographic purification of the diffusates been carried out the response to the diffusates

might have been greater, for the turions at this time may have contained growth inhibitors. The response may thus be a net response.

DISCUSSION: As mentioned in the method section a fault in the method invalidates the results. Were the experiment repeated sterile turions would be used and chromatographic purification of the diffusates would have been carried out. Some auxin activity may be indicated however even in the above experiment, for there should have been little difference between the angles expected from the controls and test plates, when in fact the angles were quite different.

GROWTH INITIATION OF THE TURIONS

The initial growth response of the turions occurs at temperatures between 6-10°C, and is a very small extension of the stem, with a reduction in the specific gravity of the turions (see plates 16-7). Plate 2 illustrates the two initial growth responses of the turions, which are determined by the orientation of the turion with respect to the horizontal plane.

Implicit in the study of the initial growth of the turions is an understanding of the nature of the rest period - whether it is true dormancy, or whether the rest period is environmentally imposed. Two main factors environmentally may control the rest period of the turions - temperature and light.

The turions are formed in early September, and abscissed in late September, when the temperature of the water in the lake is about 10°C. The turions do not grow after this abscission in the lake, yet if they were removed from the lake and transported to the laboratory, the turions could be induced to grow by holding them at temperatures above 5°C. The turions may have been exposed to an 'activating' light regime at the surface of the water on collection. The turions were then collected underwater in darkened plastic bottles, and transported in cooled containers, and were still able to grow at temperatures above 5°C in the laboratory. This indicates perhaps that other environmental factors may be involved in growth initiation. It was noted that in the laboratory turions remained in a state of rest for a longer period of time, if they were aerated. Despite the possible involvement of other

environmental factors the roles of light and temperature are considered only here.

There appeared to be no requirement for a period of low temperature for turions could be induced to grow by holding them at temperatures above 5°C during any part of the rest period. This contrasts with other reports concerning the rest periods of other aquatic plants. Kummerow (1958) reported that the winter buds of Hydrocharis morsus-ranae required a period of low temperature to initiate growth, and likewise Frank (1966) reported that the turions of P. nodosus require a period of low temperature to break their dormancy.

Many of the turions overwinter in the mud, or under plant debris and if light were a pre-requisite for growth, then the turions would not develop. It is shown experimentally that turions may begin growth (see plate 18) and may grow for some time (figure 8) if buried. Winter buds of Hydrocharis morsus-ranae are reported by Terras (1900) to have a specific light requirement for germination. Yellow and orange light were required for growth activation, and he found the light effect to override the temperature effect.

That the turions could be induced to grow at temperatures above 5°C any time during the rest period suggests that the rest period is largely environmentally imposed. However, the turions do not grow in the lake during the early autumn, when the temperature and daylength are similar to that of the early Spring when the turions begin growth. This indicates either that some other environmental factor is of overriding importance or limiting growth; or that there is some requirement for a rest period.

Much of the reported work on changes in endogenous hormones

during rest periods of plants has come from work with plants which have truly dormant periods. The above paragraph indicates that there may be a requirement for a rest period. The following experiments look at the effect of supplying various hormones in solution to the resting turions in an attempt to accelerate or induce growth. It is appreciated that growth initiation will be dependent on many facets of metabolism which are temperature dependent - membrane properties protein synthesis, carbohydrate metabolism, and enzyme action, and what the following experiments attempt to show only is whether these hormones may possibly be <u>involved</u> in growth initiation.

PLATE 16. Growth response of turions at 20°C with 18hr. illumination per day.

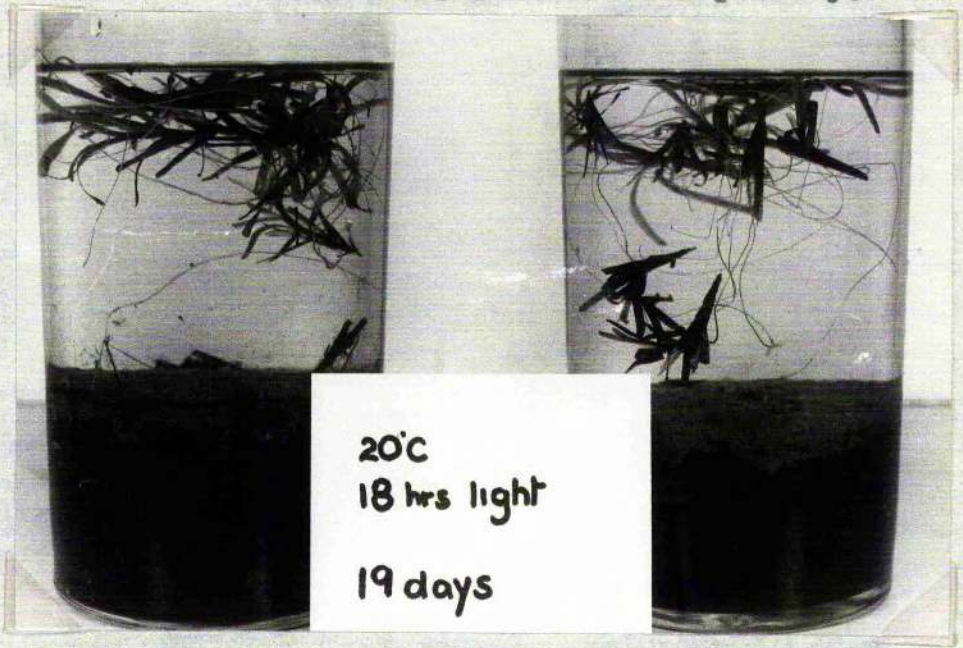


PLATE 17. Reduction in the specific gravity of the turions at the onset of growth.

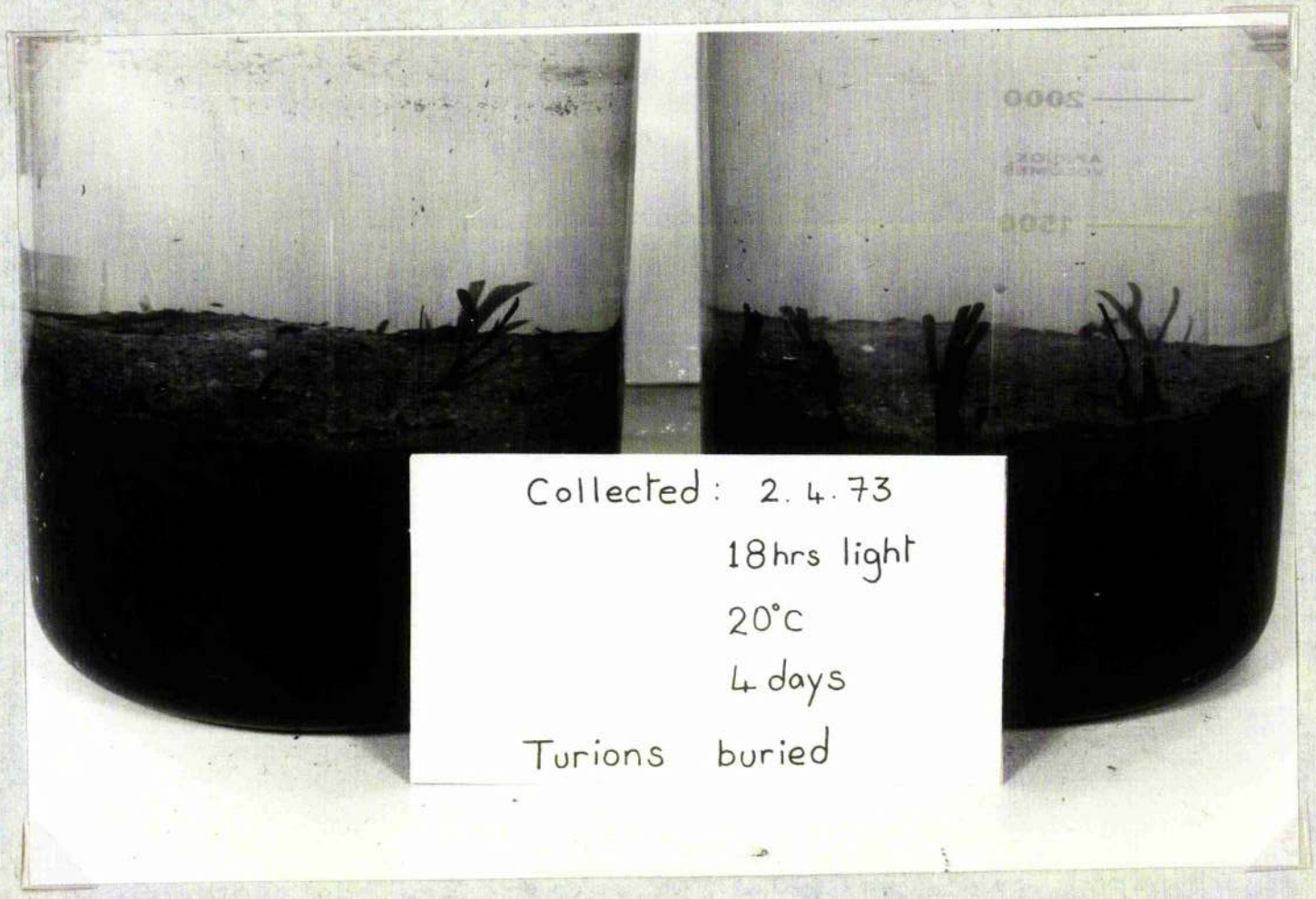


PLATE 18. Ability of the turions to grow even when buried by sediment.

PLATE 19. Geotropic response of turions and later response to light, at 20°C.

PLATE 20. Effect of high light intensity on direction of growth. Turions grown at 20°C with 18hr. illumination provided by 3 gro-lux tubes.

Stewart (1969) later similarly showed that ABA could induce turion formation in Lemna polyrhizza. Whether ABA is formed in vivo and under turion-inductive conditions has not, however, been demonstrated.

Musgrave et al (1972) demonstrated that in the amphibious Callitriche platycarpa growth promoted by submergence was a result of the stimulatory effect of ethylene accumulation. Using AMO 1618 pre-treatment (inhibits biosynthesis of gibberellins) they also demonstrated that gibberellin synthesis is a pre-requisite for ethylene-induced growth. If this system operates in Potamogeton obtusifolius M&K then temperature limitation of gibberellin synthesis or turn-over would also limit growth. Furthermore the biosynthesis of ethylene from one of its possible precusor amino acids, methionine, is enzymatically controlled. Pieterse et al (1971) isolated endogenous gibberellins from Wolfiella floridiana whose life cycle is also characterised by a turion phase, with reduced air spaces, and starch accumulation. Chromatographic purification defined two zones of gibberellin activity which they called factors 1 and 2 of which factor 1 was chromatographically similar to GA3. Comparing the concentrations of the two factors in resting and active plants they found that per unit fresh weight of tissue, the active, floating phase had eight times the amount of factor 1 that the turion phase had. The ratios of the two factors in each phase were different, suggesting possibly synthesis of active gibberellins from conjugated or inactive forms. The turions of Potamogeton obtusifolius M&K may also be so inhibited. If temperature stimulates gibberellin production

through de novo synthesis or release from conjugated forms, then one major controlling role of temperature would be defined. That the metabolsim of gibberellins is temperature dependent is demonstrated in the section devoted specifically to stem extension.

The action of gibberellic acid in growth initiation is suggested by the probable requirement for the mobilisation of starch into soluble carbohydrates at the onset of growth. This could have been tested for using ground up storage tissue. The de novo synthesis of a- and B- amylases by gibberellic acid is essential for starch degradation, and such information is well documented. Ethylene has been reported both to stimulate and to depress amylase production. Scott and Leopold (1967) reported that in lettuce hypoctyls, ethylene depressed GA3 induced a-amylase production, whilst Jacobsen (1973) reported that gibberellic acid and ethylene synergistically removed ABA-induced repression of a-amylase synthesis in barley aleurone layers. For aquatic plants, where growth is a GA3-ethylene response, it is likely that ethylene and gibberellic acid synergistically degrade starch reserves.

There is evidence for ethylene-stimulated auxin transport in aquatic plants (Musgrave et al 1973) - in Ranunculus sceleratus petioles.

If the turions are vertically orientated at the onset of growth then there can be no geotropically or phototropically induced differentials with respect to sensitivity, or concentration of, auxins, gibberellins, or ethylene, and vertical stem growth results. This explains the vertical growth that follows the geotropic curvature.

GEOTROPISM

The stem of P. obtusifolius bends through 90° if held in the horizontal position in the dark or in the light. Fig. 8 illustrates the responses of the turion. The only evidence that this is in fact a geotropic response is that the horizontally orientated turions respond in the dark (d in fig. 8). If held in the vertical position in weak light (a) or when buried, and therefore in the dark, then the stem simply elongates. This difference in the responses of the turions when held in different orientations suggests the establishment of differentials in the concentration of, or sensitivity to, growth hormones in the upper and lower halves of the geotropically stimulated turion.

Unequal auxin concentrations were for a long time quoted to explain the different growth rates of the two sides of geotropically stimulated tissue. Phillips (1972) investigated the distribution of gibberellins in geotropically stimulated green shoot tips of Helianthus and found the ratio to be 9:1, whilst Railton & Phillips (1973) found the ratio in the lower to upper halves to be 4:1 in stimulated coleoptiles of Zea mays. Furthermore, El Antably et al (1974) who initially quoted the above instances, found higher concentrations of gibberellins in the upper halves of geotropically stimulated roots of Vicia faba. Thus there is a need to assess the relative contributions of gibberellins and auxins in geotropic growth responses. Finally differentials in ethylene have also been reported in geotropically stimulated organs (Abeles 1973).

The following pilot experiments attempt to discover whether gibberellic acid and/or IAA are involved in the

geotropic growth response of the turions. Time limitation did not unfortunately allow the critical bioassay of the upper and lower halves of the geotropically stimulated turions for auxins and gibberellins. In a preliminary experiment, not described below, where gibberellic acid and IAA were applied to the lower side of the turions in 100% humidity there was acceleration of the response.

TURION RESPONSES

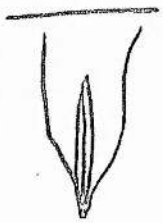
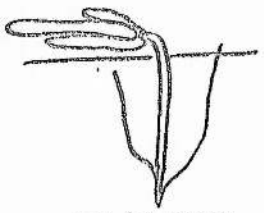
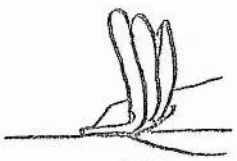
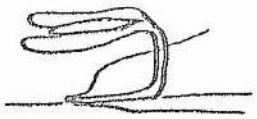
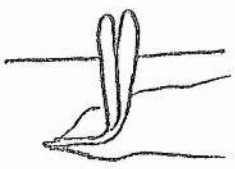
ORIENTATION	CONDITIONS	RESPONSE	MONTH	
 planted (14.1.74)	40cm water 20°C/weak light	 vertical stem elongation. (29.1.74)	JANUARY	a
 buried (14.1.74)	as above.	 rapid stem elongation in the mud. Stem then grew horizontally.	JANUARY	b
 (14.1.74)	as above.	 (29.1.74)  (30.1.74)	JANUARY	c
 buried. (2.4.75)	20°C	 (6.4.75)	APRIL	d

FIG. 8 TURION RESPONSES

EXPERIMENT 5

AIM: To investigate the effect of supplying various hormones to the turions of P. obtusifolius M&K.

MATERIALS: Turions were collected from the Lake of Menteith on April 1st, 1974.

METHOD: The compounds used were IAA, GA3, sucrose, and D(+) ribose. Sucrose was supplied to the turions in an attempt to accelerate growth because in many terrestrial plants it is the main translocated carbohydrate. Ribose was used because of its involvement in protein synthesis where growth is beginning in plants. Twelve turions were held in 250ml. of the following bathing solutions for 24hr.:

Sucrose 100-100ppm.; Ribose 100-100ppm.; IAA 100-100ppm.; GA3 100-1000ppm. by weight, and the controls were held in the same volume of distilled water. After this period 6 turions were removed from each bathing solution, washed in distilled water, and transferred in pairs to 100ml. conical flasks containing distilled water held at 5°C. The constant temperature bath was held at 5°C as this was the temperature of the loch at this time. Coupled Tecam dip coolers and heaters maintained a constant temperature. An 8hr. day was chosen, as this is approximately the daylength of early Spring. The turions were illuminated by 3 four foot 40watt gro-lux tubes, suspended 40cm. above the water level. The remaining turions were left in the bathing solutions for a longer period of time to determine whether the response of the turions, to the different compounds was different with a different exposure time, and the results are presented in table 11. Growth was scored as having occurred if the central bud of leaves of a turion just left the horizontal position.

TREATMENT ppm. by wt.		4.4.73	9.4.73	17.4.73	17.5.73
SUCROSE	100	-	-	-	-
	200	-	-	1	1
	400	-	-	-	-
	600	-	-	1	1
	800	-	-	-	1
	1000	-	-	-	-
RIBOSE	100	-	1	1	1
	200	-	-	-	-
	400	-	-	-	-
	600	-	-	-	-
	800	-	-	-	-
	1000	-	-	-	-
IAA	100	-	-	1	1
	200	-	-	-	3
	400	-	-	6	6
	600	-	-	5	6
	800	-	-	-	6
	1000	-	-	2	4
GA3	100	-	-	6	6
	200	-	-	4	6
	400	-	-	4	6
	600	-	1	6	6
	800	-	2	5	5
CONTROLS	1	-	-	-	-
	2	-	-	-	-
	3	-	-	1	1
	4	-	-	-	1

TABLE 10. Induction of growth response of turions by various substances. Turions were held in hormone solutions for 24hr. period, then transferred to water in conical flasks. (see plate 21). There were six replicate turions per treatment. Above tabulated numbers are numbers of turions.

conc.	SUCROSE	GA3	RIBOSE	IAA	CONTROLS
100ppm	2	6	5	6	4
200	3	6	4	6	
400	2	6	2	-*	
600	4	6	-	*	
800	4	6	-	*	
1000	6	6	3	*	

TABLE 11. Number of turions responding to bathing solutions (10.5.73) 36 days exposure to (6 turions/250ml.)

*Red precipitate formed with IAA 400ppm-1000ppm (effect of light)

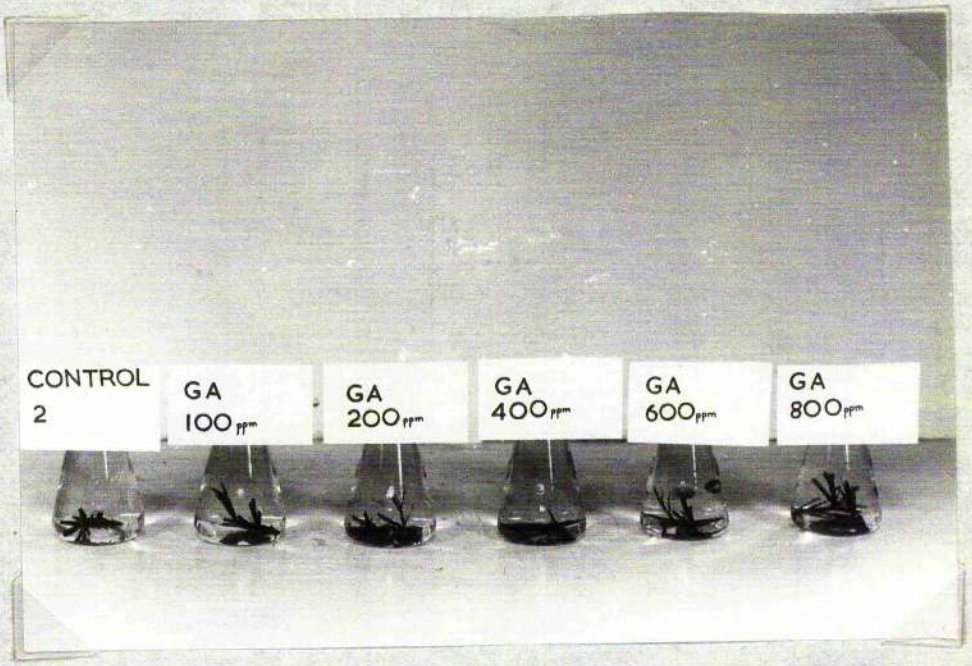


PLATE 21. Response of turions to gibberellic acid.
[Transferred to water after 24 hr. period]

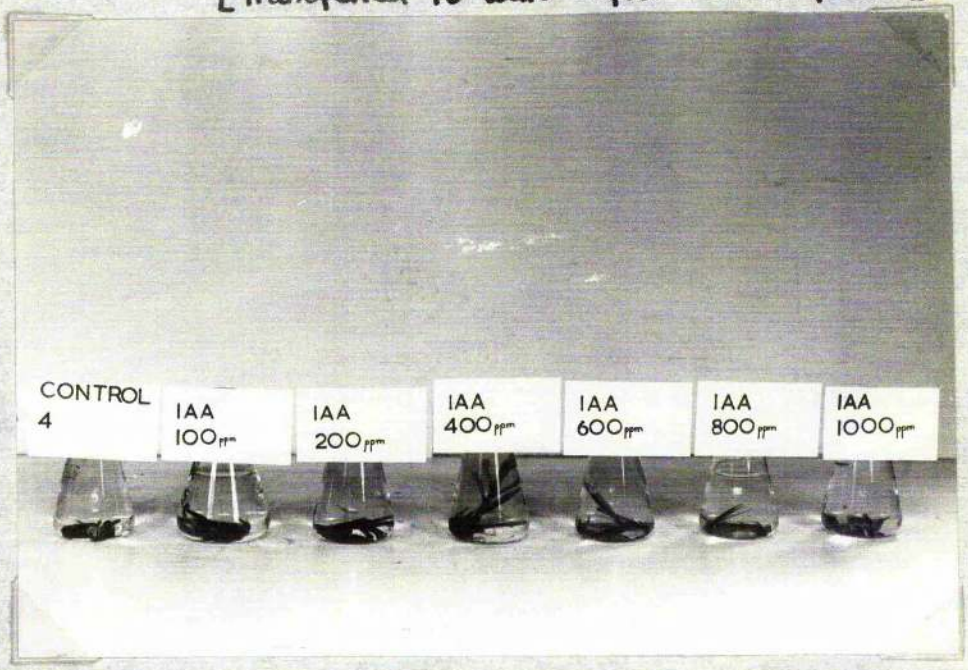


PLATE 22. Response of turions to indole-3-acetic acid.

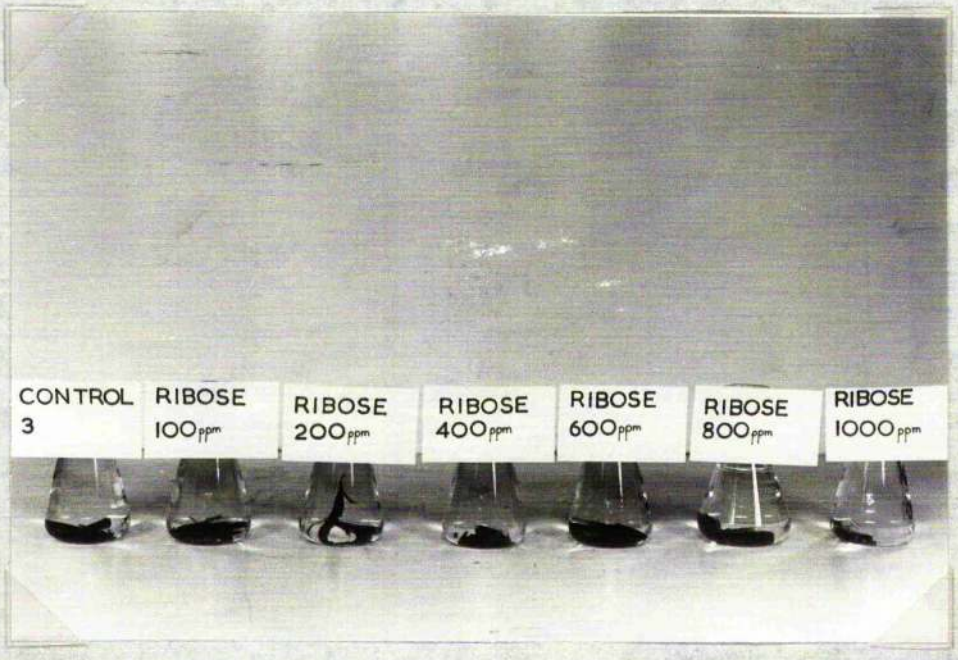


PLATE 23. Response of turions to ribose.

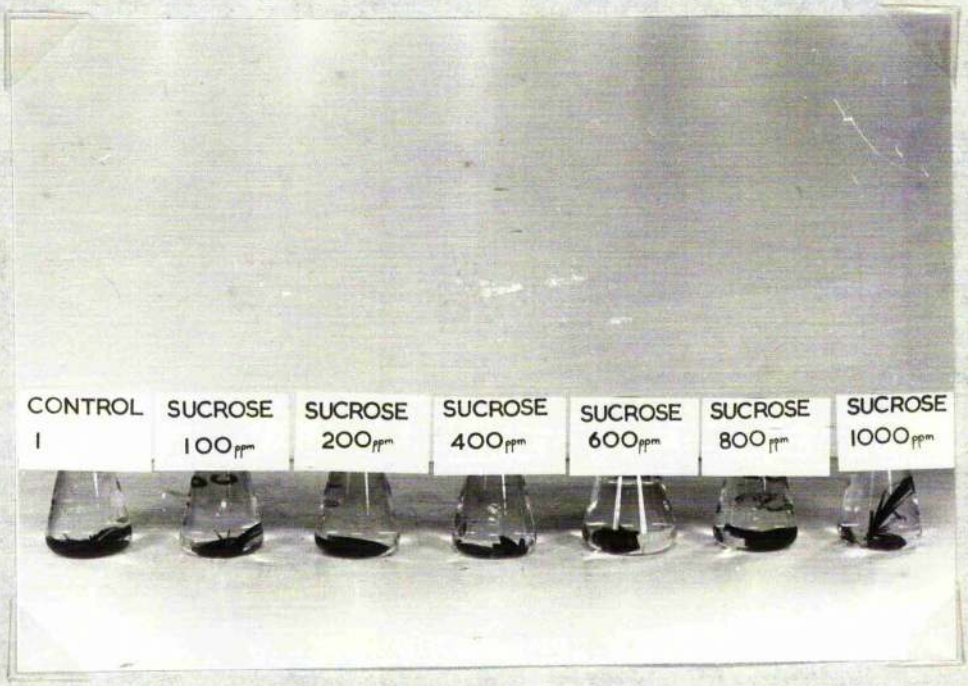


PLATE 24. Response of turions to sucrose.

RESULTS: See table 10 and 11, and plates 21-4.

1. Gibberellic acid and IAA both accelerated the rate of the growth response. Gibberellic acid initiated the most rapid response, the response occurring in several turions after 4 days, which is equivalent to a control response at 20°C.

2. Ribose and sucrose had little effect.

3. Where turions were exposed to the bathing solutions for a longer period of time (see table 11), gibberellic acid and IAA again accelerated the growth response. Sucrose at the highest concentration accelerated the growth response whilst ribose had no clear effect.

DISCUSSION: The interpretation of the results is limited by lack of data concerning the rates of penetration of the above compounds into the tissues of the turions under the experimental conditions, and also concerning the levels of endogenous hormones. Furthermore the concentrations used are very high, about 10^{-4}M. However the growth response to such solutions was normal and thus aquatic plants may have high concentrations of hormones present during the rapid bursts of growth that they exhibit. The turions were by this time beginning growth in the loch, and thus the stimulatory effects of gibberellic acid, and IAA recorded above are only acceleration of this growth.

Since gibberellins and possibly auxins are present in the turions and supply of these hormones accelerated the response, then they may be involved in the early stages of growth of the turions. Other factors may initiate the growth, but the hormones may nevertheless have a very important role. The rapidity of the response to gibberellic acid may simply reflect a rapid rate of penetration of the tissue, for with Callitriche stagnalis McComb (1965) reported that GA3 uptake into the plant

was half saturated in 15 minutes. IAA is easily photolysed by blue and red light, and thus there may have been some breakdown in solution. The effect of the IAA may have been through an induction or stimulation of ethylene synthesis in the plants.

EXPERIMENT 6

AIM: Since promotion of growth by exogenous supply of IAA may be direct or mediated by IAA-induced ethylene, this experiment studies the response of the turions to a range of IAA solutions.

MATERIALS: Turions were obtained from the Lake of Menteith on January 9th, 1974. To make up the IAA solutions, the IAA was first dissolved in a drop of absolute ethanol.

METHOD: Five turions were placed into 250ml. beakers containing one of the following: distilled water, 10^{-10}M, IAA 10^{-8}M, IAA 10^{-6}M and 10^{-4}M IAA.

To determine if supply of hormone at 5°C could overcome growth inhibition turions were held initially at 5°C in the dark. No growth response occurred within 14 days and as this response occurs within 14 days in the loch at a low temperature it was considered that the temperature might be limiting the response to the supplied hormone. The turions were transferred to 20°C in the dark therefore, and the turions began growth within 3 days.

RESULTS: The experiment was carried out from 20.2.74 - 3.3.74. Results are presented in table 12.

TREATMENT	ANGLES	MEANS ± S.E.
CONTROLS	0,30,10,40,0	16±7.10
IAA 10^{-4}M	61,78,104,86,55	76.8 ± 5.52
IAA 10^{-6}M	25,15,15,27,51	26.6 ± 5.89
IAA 10^{-8}M	8,41,30,32,12	24.6 ± 6.31
IAA 10^{-10}M	38,27,53,24,0	28.4 ± 7.81

't' test on means differences indicates that only 10^{-4}M/litre IAA had a significant effect on the growth response.

TABLE 12. Growth response of turions of P. obtusifolius M&K (Angles of central bud from horizontal) when held in bathing solutions of IAA at 5°C in the dark for 12 days, followed by transfer to 20°C in the dark.

DISCUSSION: In experiment 5 at 5°C when the turions (12 per 250ml.) were exposed to IAA bathing solutions for 24hr. and then held at 5°C and 8hr. illumination, the growth response occurred after 11-13 days. In this experiment 6 turions were held in 250ml. IAA bathing solution at 5°C for 12 days, but in the dark and no response took place. Penetration of the tissue is unlikely to have taken this time, but transport of auxin and assymetric redistribution may however have been rate-limited by the low temperature. Transfer of the turions, still in the dark, to 20°C, initiated the response within 3 days, both in the controls and in the turions exposed to IAA bathing solutions but controls kept at 5°C in the dark had still not responded. This indicates that even were sufficient auxin present endogenously, at 5°C the response would be very slow. Since the gross visual growth response occurred within 3 days at 20°C the cellular response may have been immediate. Furthermore since 10^{-4} M IAA was required to enhance the growth response at 20°C, ethylene may have been induced. IAA stimulation of ethylene synthesis was reported by Pratt & Goeschl (1969) and by Sargent et al (1974). Sargent and co-workers have found that in Pisum sativum 3×10^{-3}M IAA causes an 11-fold increase in ethylene production, with maximum after 9hr. This hypothesis could have been tested by supplying metered ethylene to the turions or by holding them in ETHREL E. This was not carried out at this stage in the thesis, for neither the theoretical implications of ethylene in growth, nor its use experimentally were known in sufficient detail

Changes in the soluble carbohydrates in the turions in the early phases of visible growth are considered in the following experimental analyses.

Soluble carbohydrates

(a) Introduction. Carbohydrates have very many uses in plants. Ribose is a component of nucleotides, whilst pentoses arabinose and xylose are common constituents of cell wall polysaccharides. Glucose and fructose are involved in sucrose synthesis. β-glucose units are the basis of the cellulose molecule, whilst α-glucose molecules are the basis of the starch molecule. The hexitol, m-inositol, is the initial metabolite in pectin formation, via glucoronic and galacturonic acids. The two main disaccharides occurring in the free state in plants are sucrose and maltose. Under conditions of decelerating growth, soluble carbohydrates may be converted to storage products such as phytic acid (from inositol) and starch (from soluble carbohydrates).

Thus there are adequate general reasons for studying soluble carbohydrate levels in plants. The levels of carbohydrates in aquatic plants were considered to be worth investigating, to determine:

a. ~~Whether~~ If there was a large fluctuation in any of the soluble carbohydrates at the onset of growth of the turions of P.obtusifolius M&K

b. ~~Whether~~ If there was partitioning and preferential use of certain sugars by various parts of the plants at different times of the year.

Initially considerable time was spent analysing extracts from plants of the following species collected in situ: P. crispus, Myriophyllum spicatum, Littorella uniflora, Chara sp., Nitella sp. and Potamogeton obtusifolius M&K. The chromatograms presented are a selection of the most meaningful ones, with

regard to interpretation of the remainder of the thesis, and most of the extracts are thus from P. obtusifolius M&K, the life cycle of which is studied in detail. Many hormones are known to control the induction and secretion of enzymes required for sugar formation, and for interconversion of sugars. An attempt is made in this thesis to relate changes in the carbohydrate levels with hormonal levels.

Soluble carbohydrates were analysed using GLC analysis. This was chosen in preference to the lengthy anthrone technique. The anthrone technique, which is a spectrophotometric estimation, is more suitable for use when only a measure of total soluble carbohydrate is required. Using GLC, sugar derivatives appear individually as peaks on the GLC trace, which allows estimation of both the individual sugars, and the total soluble carbohydrates.

To study the soluble carbohydrates in plants of P. obtusifolius M&K, whole turions and plants were extracted each month from material collected between January and September 1973. As the ratio of plant parts varies throughout the growing season, it was difficult to relate variations in the sugar levels within the whole plants, to a particular phase of development. Plant parts, rather than the developing whole plants should have been analysed on a seasonal basis. Thus the data presented in this chapter concerned with growth initiation are the only data on the soluble carbohydrates that are presented. Using co-chromatography the main sugars found in the turions were fructose, α- and β-glucose, mannitol, m-Inositol and sucrose. No maltose was found in the turions or plants, despite the abundance of starch reserves, and thus the α-glucose detected must represent the decomposition product of the starch reserves.

Soluble carbohydrates : Glossary

The following abbreviations are employed:

TMS - Tri-Methyl- Silyl

TCMS - Trichloromethylsilane

HMDS - Hexamethyldisilizane

M - Mannitol

I - m-Inositol

F - Fructose

α-G - α-Glucose

S - Sucrose

β-G - β-Glucose

GA3 - Gibberellic acid

IAA - Indole-3-Acetic acid

GLC - Gas liquid chromatography

(b) Extraction of soluble carbohydrates. When collected from the loch or on removal from experimental conditions plants to be analysed for carbohydrates were added to boiling tubes containing 80% ethanol. This volume was later made up to 25ml.. The carbohydrates were exhaustively extracted in hot ethanol. Two further extractions with 10ml. 80% ethanol and two of 10ml. 60% ethanol were made, and the combined extracts were added to a pre-weighed, round-bottomed, evaporating flask, and dried down to a gum using a rotary evaporator. The water bath temperature for the evaporation procedure was 40°C. On evaporation of the extracts the flasks plus gums were weighed. Total dry weight of tissue was then taken as the sum of the weight of the gum plus the oven dry weight of the extracted tissue. Ethanolic extraction of plant tissue, results in the removal of Kreb's Cycle acids, amino acids, fats, lipids and carbohydrates. The resulting chromatogram from such an extract is called a 'metabolic profile' and an example of this is illustrated in fig. 9. page 69 To determine whether peaks from such chromatograms tentatively identified (by co-chromatography) were in fact carbohydrates the following purification procedure was developed with the assistance of Dr. Brian J. Knights, Carnegie Research Laboratories, Glasgow. For routine analysis, the procedure was not adopted, however, as it proved too time-consuming.

(c) Purification of ethanol extract. As the technique was not used in most of the following work, this purification technique is summarised. After exhaustive extraction with ethanol, the tissue was removed, and the samples were shaken

up with a suitable volume of 40:60 petroleum ether, to remove fats and lipids present. The samples were then evaporated down to a small volume, and then passed through an Amberlite anion exchange column using distilled water as the eluent. Kreb's cycle acids present are retained on the column, and the column was regenerated with 2N ammonium hydroxide. The sample was then evaporated down once more, and passed through a Zeo-Karb 225 cation exchange column, which retains amino acids present in the extract. This column was regenerated by washing with 0.1N HCl. Again the eluent used was distilled water. The sample now contains 'only' carbohydrates and neutral amines. The procedure is summarised below:

Ethanol extract	Removed
40:60 petroleum ether	Fats, lipids
Amberlite anion exc.	Kreb's cycle acids
Zeo-Karb 225 cation exc.	Amino acids
Carbohydrates (plus neutral amines)	

Figure 9 illustrates an extract from the leaves of E.orlonus, but the extract in figure 10 has been subjected to the above purification. Although at a lower attenuation (see figs. for details) the extract in figure 10 has undergone obvious purification. That the same main peaks a-g appear in this purified extract, strongly supports the tentative identification of the peaks in the crude ethanol extract of this species.

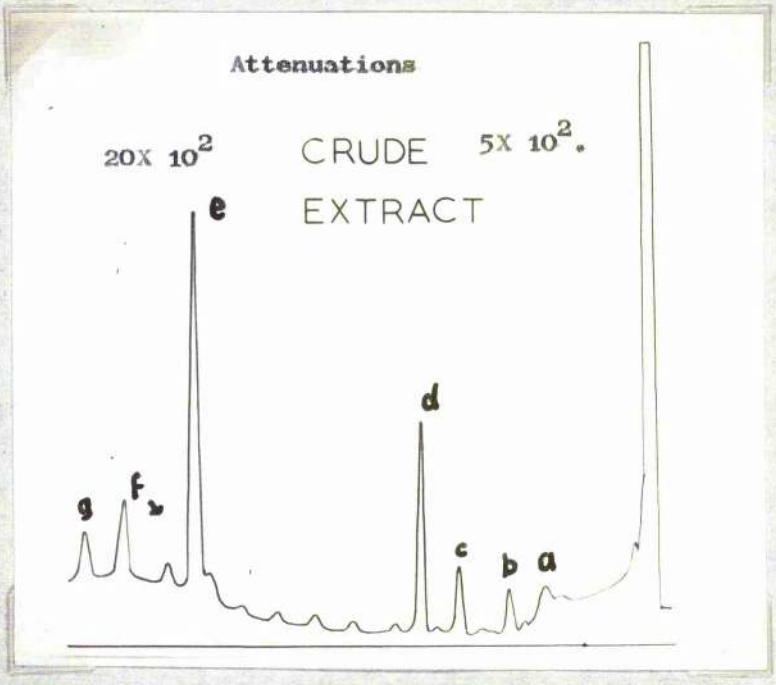


FIG. 9 Chromatogram of crude ethanol extract from leaves of P.crispus, 0.37 fresh wt. Temperature programme - 6°C per min.

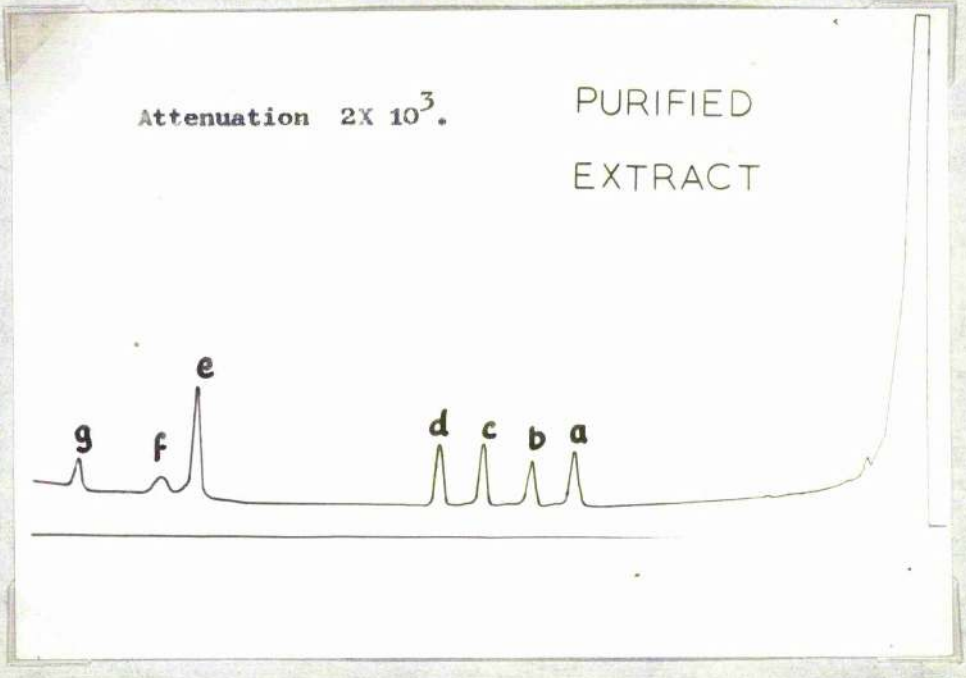


FIG. 10. Chromatogram of purified ethanol extract, from leaves of P. crispus. 1g. fresh wt. Temperature programme - 6°C per min.

(d) Formation of sugar derivatives and GLC procedure. TMS* derivatives of the soluble carbohydrates were made. The preparation of TCMS* detivatives was first made by Sweeley et al (1962). TMS derivatives of most sugars give single peaks on analysis by GLC. The technique of GLC of soluble carbohydrates and the problems incurred are discussed in detail by Holligan & Drew (1971).

The gum prepared by the above extraction technique was redissolved in 1ml. dimethyl sulphoxide. Water is excluded at all stages in the formation of the derivatives, as the column is rendered inefficient if a sample containing water is injected. Coagulation of the precipitate in the reaction mixture indicates contamination by water. A 0.2ml. aliquot of the redissolved extract was added to a small sample tube. To this was added sequentially 0.2ml. Hexamethyl disilizane, and 0.1ml. TCMS. The tube was quickly re-stoppered, and the reaction mixture was shaken vigorously for 1 minute. Two phases separate out, and it is the upper phase that contains the carbohydrate derivatives. It is from this phase that micro-quantities are removed for injection into the GLC.

The micro sub-sample was injected into a basic Pye 104 Series Chromatograph with a flame ionisation detector, which was coupled via an amplifier to a Phillips PM 8220 Pen recorder. To eliminate as many variables as possible a standard set of GLC conditions were adhered to:

* See Glossary, page 66.

COLUMN: Glass, 5ft. in length, ⅛" Diam.

PACKING: Diatomite C 'Q' 60-70 mesh, with a 2% loading of silicone gum SE 52.

SYRINGE: Hamilton 11cm. needle.

TEMPERATURE PROGRAMME: 100-270°C @ 6°C per minute.

AMOUNT INJECTED: 2-8µl.

FLOW RATES: Nitrogen - 30ml.min^{-1}, Hydrogen - 15lb.in^{-2}, Air - 15lb.in^{-2}

PAPER SPEED: 2cm. per min.

ATTENUATION: 20 X 10^2.

(e) Quantification of soluble carbohydrates. Peak areas can only be related to absolute amounts of individual sugars by the injection of known concentrations of each sugar under identical GLC conditions. It is generally incorrect to assume that equal amounts of different substances produce equal peak areas, because of differences in thermal conductivity and molecular weights. Sugars were dissolved in 1ml. dimethyl suphoxide (as were samples) and a 0.2ml. aliquot of this was used to produce the sugar derivatives. The peak area obtained from injecting 2µl from the reaction mixture was then referred back to this original weight of sugar in the 1ml. dimethyl sulphoxide. Standards used were fructose, α-glucose, mannitol, β-glucose, m-Inositol, and sucrose. With the concentrations of sugars used there was no visual difference in the volume of the upper phase of the resulting reaction mixture. Table 13 page 74 lists the areas of the peaks, calculated by triangulation, (½ base X altitude). For each of the sugars/

sugar alcohols under the standard GLC set of conditions figure 11 page 73 illustrates the calibration graph obtained which defines the relationship between the injected sample and the weight of sugar in the original 1ml. dimethyl sulphoxide. Lines of best fit were determined using regression analysis.

REAGENTS:

Diatomite C 'O' 60-70 mesh	Pye Unicam Gas Chromatography Mat.
Methyl Phenyl Silicone Gum SE52	"
Trichloromethylsilane (TCMS)	BDH
Hexamethyldisilizane (HMDS)	"
Dimethylsulphoxide	"
Amberlite Resin IR 4B(OH)	"
Zeo-Karb 225 SRC 13	" (Permutit)
Fructose	"
D Glucose	" (Microanalytical)
Meso-Inositol	" (Biochemicals)
Mannitol	" "
Maltose	BDH (lab.)
D(-) Ribose	" (Biochemicals)
Sucrose	" (Analar)

In Experiments 7, 8 and 9 standards were coinjected with each sample and peak indentification based on this co-chromatography.

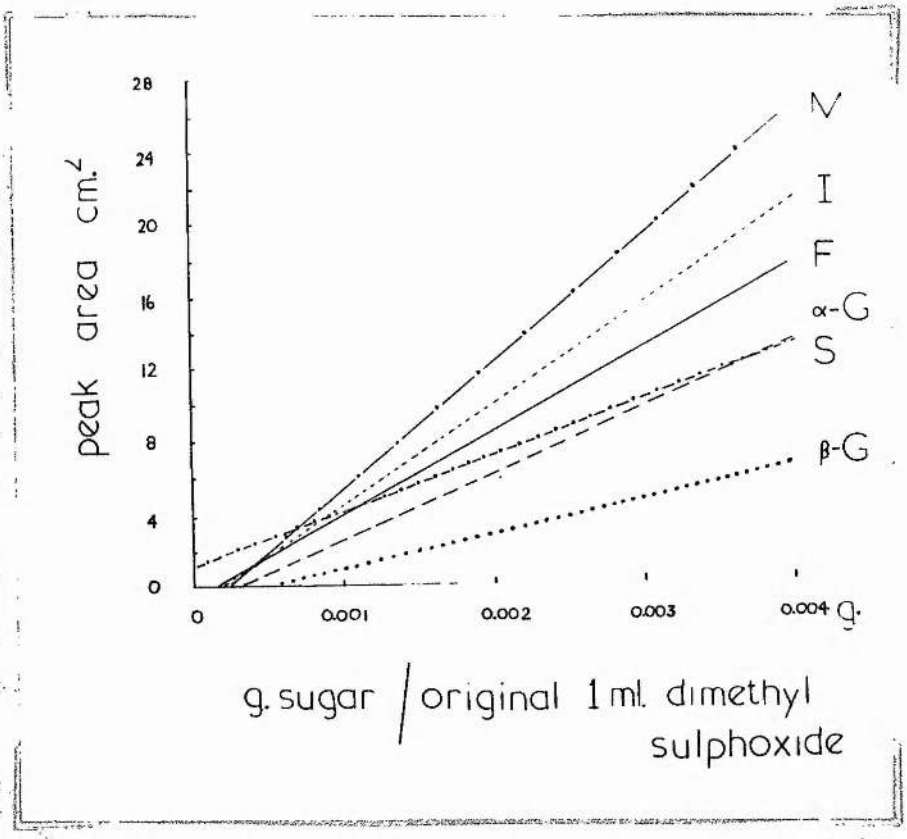


FIG. 11. Calibration graph for sugars and sugar alcohols.

LEGEND: M-Mannitol

I-Inositol

F-Fructose

α-G - α-Glucose

S-Sucrose

β-G - β-Glucose.

See glossary (page 66) in future.

REFERENCE CONCENTRATION	AREAS OF PEAKS (cm^2) FOR 2μl INJECTIONS FROM THE DER. OF 0.2ml ALIQUOT					
gm. sugar per ml. dimethyl sulphoxide	FRUCTOSE	-GLUCOSE	MANNITOL	B-GLUCOSE	m-INOSITOL	SUCROSE
0.0005	2.17±0.07(2)*	1.82±)(2)	2.28±0.17(2)	0.98±0.19(2)	3.11±0.39(2)	1.45±0.05(2)
0.001	3.54±0.08(5)	1.77±0.06(5)	4.94±0.04(5)	0.99±0.07(5)	5.20±0.19(5)	4.53±0.16(4)
0.002	7.36±0.24(2)	5.43±0.35(4)	10.96±1.81(4)	1.90±0.10(4)	7.66±0.11(5)	9.24±1.00(2)
0.004	17.81±1.87(3)	13.91±0.96(2)	26.56±1.92(2)	6.95±0.31(3)	23.04±0.78(4)	12.43±0.62(3)

* Figures in brackets are no. of replicate injections from same sample.

TABLE 13. Relation of area of peaks of sugar standards to conc. of sugar in original 1ml. Dimethyl Suphoxide.

74.

EXPERIMENT 7

AIM: Plates 2-3 illustrate that during this period growth occurs on a measurable scale. It was thus decided to analyse the turions for soluble carbohydrates just before growth had begun (March 2nd) and also when obvious growth was resuming (April 18th). Turions were collected from the Lake of Menteith on these dates, and were added to 80% ethanol for carbohydrate analysis, as described above. The whole turions were extracted.

RESULTS: Tables 14 and 15 (pages 76 and 77 respectively) present the data for the GLC analysis of the sugar derivatives of the extracts. Table 16 (page 78) lists the concentrations of sugars/sugar alcohols in the two extractions in mg. sugar/per g.tissue. Again no visual difference was observed in the volume of the upper phase within or between the two samples, and so was not measured. The significances of the means differences were ascertained using the students 't' test.

MARCH 2nd. 1973

SAMPLE		FRUCTOSE	-GLUCOSE	MANNITOL	B-GLUCOSE	INOSITOL	SUCROSE	TOTAL WT. TISSUE
8. Area Peak(cm^2)	(A)	2.84	0.75	1.71	0.78	1.82	1.39	
Weight(g)	(W)	0.000800	0.000525	0.000500	0.000900	0.000525	0.000125	0.0652
2.	(A)	2.48	0.63	1.71	0.61	2.10	1.40	
	(W)	0.00725	0.000500	0.000500	0.0008	0.000575	0.000125	0.0800
4.	(A)	1.33	0.42	1.26	0.48	1.61	0.93	
	(W)	0.000500	0.000450	0.000425	0.000750	0.000500	-	0.0665
1.	(A)	2.03	0.66	1.68	0.74	1.98	1.40	
	(W)	0.000600	0.000500	0.000500	0.000850	0.000575	0.000125	0.0777
11.	(A)	3.78	0.80	2.41	1.00	2.25	1.05	
	(W)	0.001000	0.000550	0.000600	0.00100	0.000600	0.000025	0.0899
12.	(A)	3.46	0.77	2.47	0.76	1.68	1.40	
	(W)	0.00925	0.000550	0.000625	0.000900	0.000500	0.000125	0.0898
10.	(A)	2.47	0.63	1.43	0.60	1.31	1.05	
	(W)	0.000725	0.000550	0.000450	0.000800	0.000450	0.000025	0.0827
3.	(A)	2.63	0.75	1.72	0.94	1.61	1.20	
	(W)	0.000750	0.000525	0.000500	0.000975	0.000500	0.000050	0.0620
9.	(A)	2.12	0.56	1.43	0.74	1.50	0.84	
	(W)	0.000625	0.000475	0.000450	0.000870	0.000475	-	0.0624
7.	(A)	3.91	1.16	2.50	1.57	2.77	1.42	
	(W)	0.001025	0.000600	0.000625	0.001300	0.000700	0.000125	0.0990
6.	(A)	2.88	1.04	2.13	1.19	2.06	1.15	
	(W)	0.000825	0.000600	0.000550	0.001100	0.000575	0.000050	0.0988
5.	(A)	1.32	0.22	1.13	0.22	0.45	0.77	

APRIL 28th, 1973.

SAMPLE		FRUCTOSE	α-GLUCOSE	MANNITOL	β-GLUCOSE	INOSITOL	SUCROSE	TOTAL WT. TISSUE
3.	(A)	0.15	0.35	3.00	0.15	1.76	2.27	
	(W)	0.0002	0.000425	0.0007	0.00055	0.000525	0.000425	0.0927
4.	(A)	0.11	0.17	1.46	0.11	0.91	1.92	
	(W)	0.000175	0.000375	0.00045	0.0005	0.000375	0.000275	0.0862
6.	(A)	0.63	0.51	1.36	0.72	0.97	0.35	
	(W)	0.000300	0.000475	0.000430	0.000850	0.0004	-	0.0648
8.	(A)	0.18	0.13	1.92	0.07	0.63	1.82	
	(W)	0.0002	0.000350	0.000525	0.0005	0.000325	0.00025	0.0870
1.	(A)	0.10	0.22	1.85	0.12	1.64	2.99	
	(W)	0.000175	0.000375	0.000525	0.0005	0.0005	0.00065	0.1336
2.	(A)	0.32	0.55	3.25	0.27	2.45	2.64	
	(W)	0.000225	0.000475	0.000725	0.000625	0.00065	0.00050	0.1010
5.	(A)	0.24	0.13	2.52	0.12	1.75	2.13	
	(W)	0.0002	0.00035	0.000625	0.0005	0.000525	0.000350	0.1000
9.	(A)	0.05	0.11	1.23	0.13	1.26	1.29	
	(W)	0.000150	0.000325	0.000425	0.0005	0.000425	0.000075	0.0791

TABLE 15. Areas of peaks and weights of sugars in original 1ml. dimethyl sulphoxide.

mg. sugar/g. tissue (MEAN)

	MARCH 2	APRIL 28	SIGNIFICANCE OF MEANS DIFFS.
FRUCTOSE	9.0 (12)*	2.0 (6)	P 0.001
α-GLUCOSE	8.0 (12)	4.0 (6)	P 0.001
MANNITOL	7.0 (12)	6.0 (6)	NS
β-GLUCOSE	12.0 (12)	6.0 (5)	P 0.001
m-INOSITOL	7.0 (12)	5.0 (6)	P 0.001
SUCROSE	0.7 (12)	4.0 (6)	P 0.001
Total SOLUBLE CARBOHYDRATE	43.7	27.0	

* Figures in brackets indicate the number of samples analysed.

TABLE 16. Changes in Carbohydrates in turions at onset of growth in Lake of Menteith.
(Period of growth = 2 months)

RESULTS: Between 2nd March and 28th April:

1. There was a marked decrease in the levels of fructose.
2. The concentration of α- glucose fell slightly.
3. There was no change in the concentration of mannitol.
4. The concentration of β -glucose fell.
5. There was a slight decrease in the concentration of m-Inositol.
6. The concentration of sucrose rose dramatically by about 5X its previous concentration.
7. The total soluble carbohydrate concentration fell.

DISCUSSION: Sucrose is formed from 1- -glucose, and

2-β-Fructofuranose. The fall in the levels of both fructose and α-glucose is reflected in a marked increase in sucrose concentration. Decrease in fructose may also be due to some conversion to mannitol, which is formed by direct reduction of fructose. For many land plants, especially fungi, mannitol may be utilised immediately as a carbon source. (Lewis and Smith 1967). The decrease in the concentration of β-Glucose may be due to cell wall formation, β-glucose molecules being the basis of the cellulose molecule.

The high concentrations of the tentatively identified m-Inositol are of interest. M-Inositol has been reported in the literature (Pollard et al 1961) to be required with IAA for growth, but at hormonal levels. The higher levels in this aquatic species may explain the later phases of growth which involve rapid elongation. Inositols are present in lipid membrane material (Hawthorne 1964), and are required in pectin formation. A complex of this sugar alcohol and IAA has been isolated by Nicholls (1967) in Zea mays., and thus it may be very closely linked with hormonal regulation of growth. The decrease in the level of inositol may have been due to such complexing. Conversion of m-Inositol to phytic acid has been reported in Wolfiella floridiana (Roberts and Loewis 1968), but this is unlikely to explain the observed decrease, for phytic acid is phosphate storage material, and thus formed therefore under conditions of decelerating growth. A more feasible explanation for the decrease in the inositol may be in its incorporation into cell wall polysaccharides as reported in Lemna and Petroselinum by Roberts and Loewis (1967). The decrease in total soluble

carbohydrate observed during this period may be a reflection of conditions of light and temperature favourable for the formation of starch reserves. The following experiment indicates that there is a rise in the total carbohydrates in the turions in the very early stages of growth. The most dramatic change to occur was the marked increase in sucrose concentration. Sucrose is the main translocated sugar in most terrestrial plants and suitably so, for this enables the easy transport of glucose and fructose residues to specific sites of growth. The relation of increases and decreases in sugars to interconversions is however only very tentative, as the results from a single extract are rather a static picture of an extremely labile carbohydrate regime.

Having ascertained that there was a drastic increase in soluble carbohydrates in the turions over this period, it was decided to determine in which tissue - stem, leaf or ligule, that the greatest increase in sucrose occurred, and furthermore whether such an increase occurred during the period of the geotropic response.

EXPERIMENT 8.

AIM: To determine the levels of carbohydrates in the turion parts before and immediately after the first visible sign of growth....the geotropic growth response. This experiment was stimulated by the work of Frank (1966) who suggested that, with P. nodosus, one of the possible roles of the bud scales might be as a carbohydrate store.

MATERIALS: Turions were collected from the Lake of Menteith on 25th November, 1973.

METHOD: Ten dormant turions were dissected into stems, leaves and ligules, and like parts bulked together, to ensure measurable quantities of carbohydrates. These bulked tissues were extracted by the normal technique, and the TMS derivatives of the soluble carbohydrates analysed under the standard GLC conditions. Similarly ten turions in which growth had just ~~begun~~ been induced were bulked and treated as above. Only one sample of dormant and growing turions was thus analysed. The extractions were made on 29th November, 1973. Results were expressed as mg. sugar per gm. tissue in table 19 (page 86).

RESULTS: Figs. 12-17 illustrate the chromatograms obtained for stem, leaf and ligule, for dormant and growing turions.

AREA OF PEAKS (2µl @ 20 x 10^2)

	FRUCTOSE	x-GLUCOSE	MANNITOL	B-GLUCOSE	m-INOSITOL	SUCRO
STEM	0.182	0.110	0.600	0.140	12.050	4.25
LEAF	0.635	0.210	1.910	0.350	1.52	5.50
LIGULE	0.030	0.030	0.16	0.045	0.360	0.71
	WT. SUGARS / ml. DIMETHYL SULPHOXIDE (FROM GRAPHS)					
STEM	0.00025	0.00035	0.0003	0.0005	0.00023	0.00
LEAF	0.0003	0.0004	0.0005	0.00065	0.0005	0.00
LIGULE	0.0002	0.0003	0.00025	0.00045	0.0002	-

TABLE 17. GLC data for DORMANT TURIONS.

AREA OF PEAKS (2µl @ 20 x 10^2)

	FRUCTOSE	-GLUCOSE	MANNITOL	B-GLUCOSE	m-INOSITOL	SUCR
STEM	0.375	0.375	0.360	0.420	12.00	18.8
LEAF	0.080	0.080	0.420	0.150	0.550	7.2
LIGULE	0.064	0.040	0.052	0.052	0.50	1.69
	WT. SUGARS (g) / ml. DIMETHYL SULPHOXIDE					
STEM	0.00025	0.00045	0.0003	0.0007	0.0023	0.00
LEAF	0.00035	0.00055	0.0003	0.00055	0.0003	0.00
LIGULE	0.00025	0.00045	0.0003	0.0007	0.0003	0.00

TABLE 18. GLC data for GROWING TURIONS.

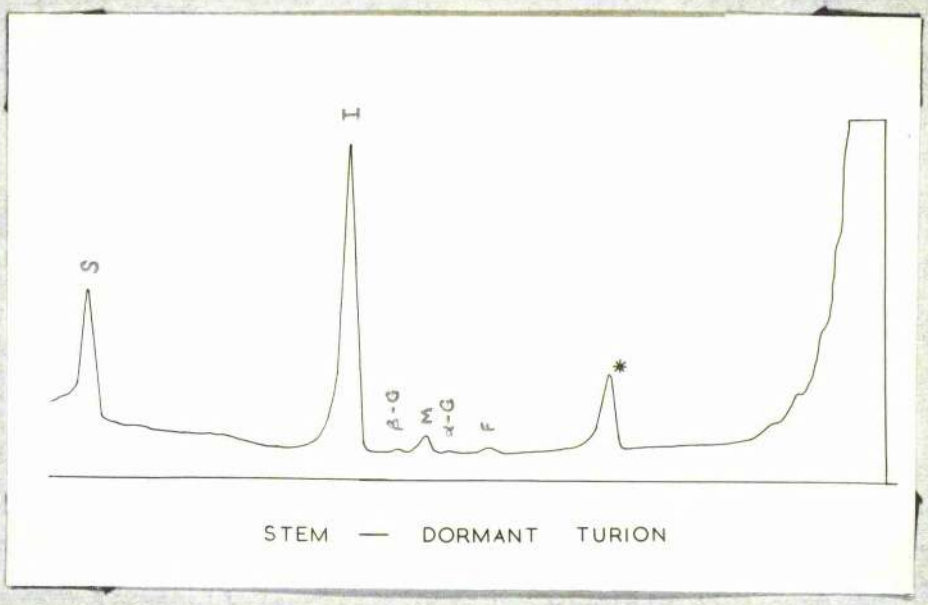


FIG. 12. Soluble carbohydrates in stem of dormant turion. *(internal standard, n-hexadecane) 4ul. injected, attenuation 20 x 10^2.
See page 73 for key to initialled peaks.
These are the main peaks in all traces and so subsequent chromatograms are not labelled.

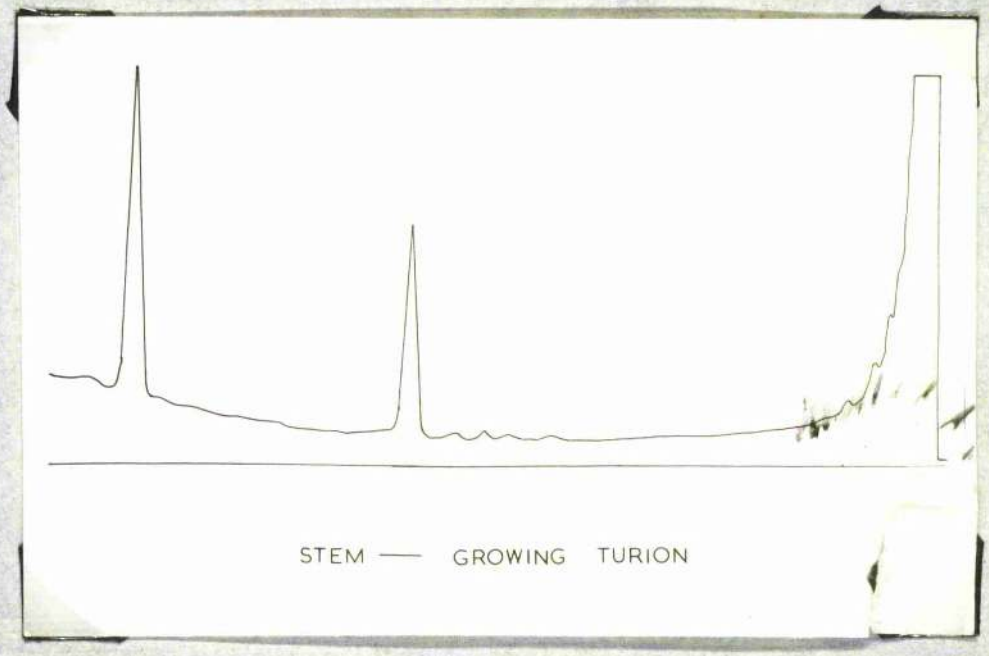


FIG. 13. Soluble carbohydrates in stem of growing turion.
2ul. injected, attenuation 10 x 10^2.

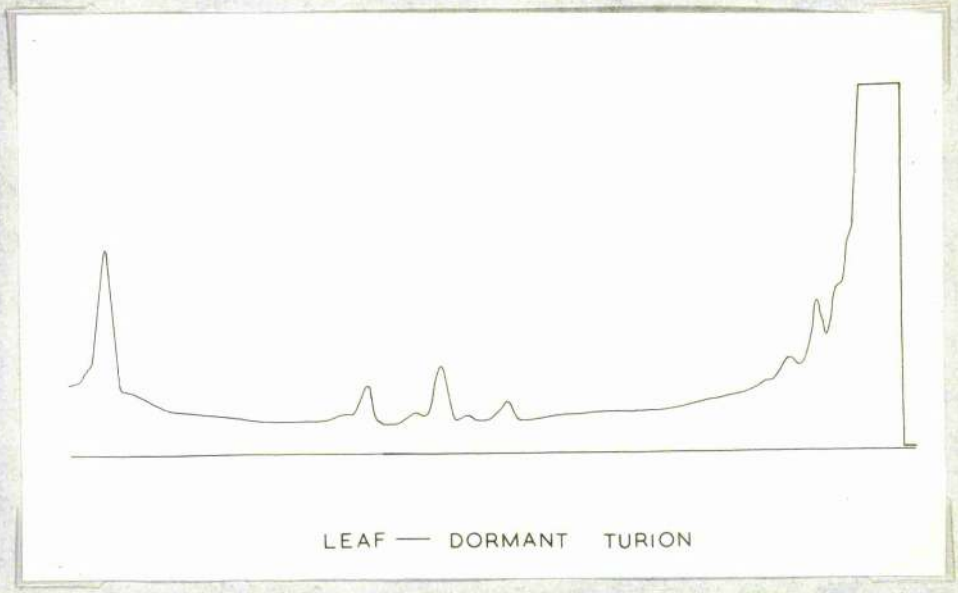


FIG. 14. Soluble carbohydrates in leaves of dormant turions.
4µl. injected, attenuation 20 x 10^2.

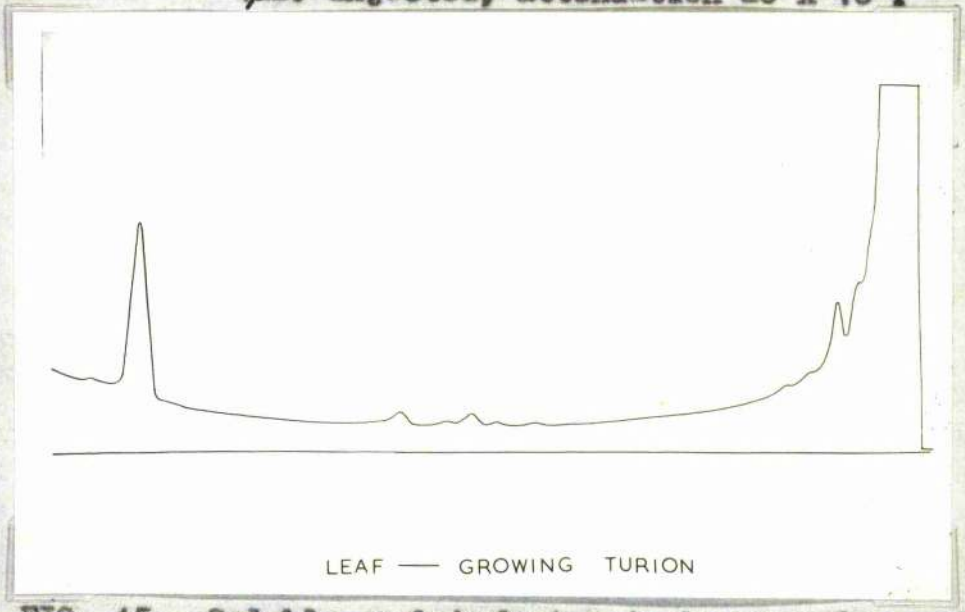


FIG. 15. Soluble carbohydrates in leaves of growing turions.
4µl. injected, attenuation 20 x 10^2.

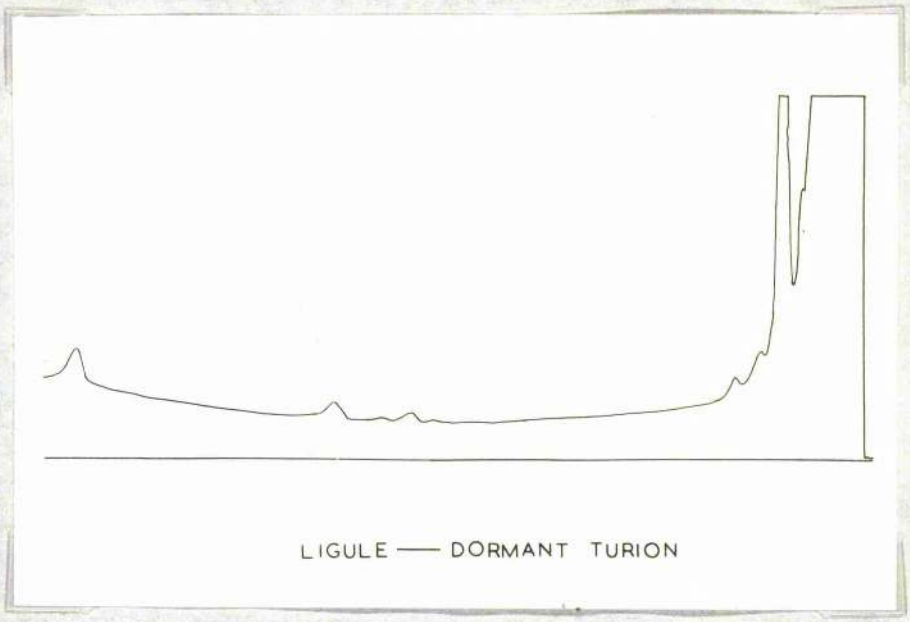


FIG. 16. Soluble carbohydrates in ligules of dormant turions
8µl injected, attenuation 20 x 10^2.

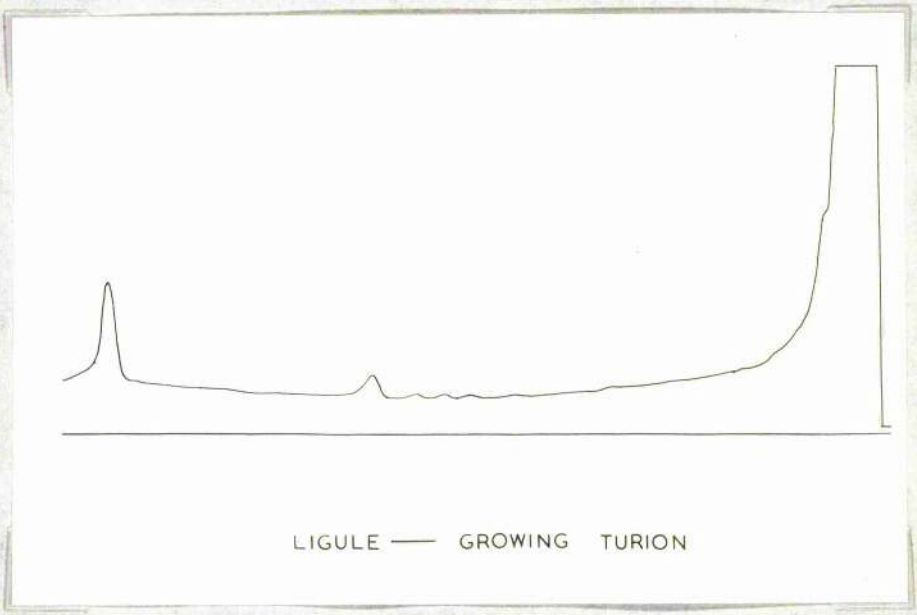


FIG. 17. Soluble carbohydrates in ligules of growing turions.
8µl. injected, attenuation 20 x 10^2.

	STEM		LEAF		LIGULE	
WT. TISSUE (g)	0.164	0.120	0.164	0.073	0.059	0.042
SUGAR	DORMANT	GROWING	DORMANT	GROWING	DORMANT	GROWING
Fructose	1.5	2.0	1.8	4.8	3.4	5.9
a-Glucose	2.0	3.7	2.4	7.5	5.0	10.7
Mannitol	1.8	2.5	2.7	4.1	4.2	7.1
B-Glucose	3.0	5.8	4.0	7.5	7.6*	16.6*
Inositol	13.8*	19.6	3.0	4.1	3.4	7.1
Sucrose	6.0	48.3*	9.1*	27.4*	-	4.8
TOTAL SOLUBLE CARBOHYDRATES (mg.sugar/g.tissue)	28.1	81.9	25.0	55.4	23.6	52.2

* Indicates principal soluble carbohydrate b weight.

TABLE 19. CHANGES IN SOLUBLE CARBOHYDRATES IN TURION PARTS AT ONSET OF GROWTH.

Results are expressed as mg. each sugar /sugar alcohol per gm. dry wt. tissue.

RESULTS: Results are presented in tabulated form in table 19 where sugar concentrations are expressed as mg. sugar/g.wt. tissue. The results from this experiment are probably more representative of the changes that occur in the turions in the very early stages of growth than are the results in experiment 7.

When the turions are analysed immediately growth is seen to have begun, the levels of all the soluble carbohydrates in all plant parts have increased. The ratios of the concentrations of the totals of their carbohydrates in the various parts of dormant and active turions are respectively and approximately stem, leaf and ligule 3:1, 2:1, 2:1 in favour of the active turions.

In the stem the principal sugar or sugar alcohol by weight is m-Inositol for dormant turions and sucrose for growing turions. This is illustrated clearly in the chromatograph presented in figs. 12-13. In the leaf the principal carbohydrate is sucrose, both in the dormant and active turions, (see figs. 14, 15, page 84 while in the ligules both in the dormant and growing turions (see figs. 16, 17), the principal sugar is B-glucose.

The sucrose concentration rose in all plant parts, but in the stem the ratios of the sucrose concentrations in the turions was 8:1 in favour of the growing turions.

DISCUSSION: Two main points come out of this analysis:

1. The ligules do not contain reserve quantities of soluble carbohydrates, and as they represent only about 1/7th of the dry weight of the turion (see ~~fig.~~ table 19)it is unlikely that their prime function is to store carbohydrates. It is thus suggested that their main function is rather to

control the gaseous regime of the leaves of the turion. When growth is beginning, the ligules are seen to 'peel away' from the leaves in the central bud, allowing the leaves to assume their new orientation (see plates 3,4) pages 9,10 Bubbles of gas appear between the leaves and ligules at this stage, and these may have been ethylene-rich. The role of membraneous coverings in controlling winter bud growth is stressed by Vegis, and reiterated by Frank (1966).

2. The sucrose level rose dramatically at the onset of growth. The geotropic response is a stem response, as isolated stems were able to respond geotropically. Since the main carbohydrate change in the stem was in the sucrose levels, it is likely that sucrose was actively involved in this early phase of growth.

Supporting this are the findings of experiment 10 that exogenous sucrose promoted stem extension under normally inhibitory conditions (page 104), and that of experiment 5 where exogenously supplied sucrose accelerated the geotropic growth response (page 57)

Two factors however prevent precise relationship of the above findings to those in the previous experiment. Firstly different periods of growth were involved, and secondly, the light regimes in the loch and under the laboratory conditions were different, and the effect of light of different spectral composition on the conversion of carbohydrates has been shown to be highly significant (Karnachuk et al 1972).

The following experimental evidence indicates that control of sucrose levels may be exerted at the hormonal level.

EXPERIMENT 9

AIM: To determine what changes occur in the soluble carbohydrates of turions exposed to bathing solutions of gibberellic acid, sucrose, and indole-3-acetic acid.

METHOD: Two turions per treatment (described in experiment 10 page 1) were removed from the bathing solution after 18hr. The turions were first washed in distilled water, and then they were placed in tubes containing 80% ethanol, to determine, using GLC analysis, whether during this period the carbohydrate regimes had been affected by the three compounds: IAA, GA_3, Sucrose. The chromatograms presented in figs. 18-22 were chosen as the turions came from bathing solutions which were later demonstrated to promote the subsequent development of the turions (see table 20, page 104)

RESULTS: Since tissues from different bathing solutions had similar dry weights, the results are presented in the forms of the original chromatograms, which are mostly at the same attenuation, thus allowing immediate comparison.

Gibberellic acid stimulated m-inositol, and the hexoses, but had little effect on the sucrose concentration (see figs. 20 and 22).

Sucrose stimulated internal levels of all soluble carbohydrates (see figs, 21 and 22).

100ppm. IAA stimulated sucrose levels, plus perhaps m-Inositol, (see figs. 18 and 22), whilst 1000ppm. caused a reduction in sucrose levels (compared to the controls and to 100ppm. IAA), (see figs, 18, 19, 22). There may also have been reduction in the levels of the hexoses and hexitols, in

response to this concentration of IAA.

DISCUSSION: The growth promotion caused by exogenous supply of (A) does not result from a stimulation of internal sucrose levels (see figs. 20 and 22), but rather gibberellic acid appeared to be more closely related with the hexose levels. Yet the main change in the carbohydrate levels was that of sucrose, and so the control must lie elsewhere. IAA at a concentration of 100ppm. increased the sucrose concentration in the turions (see fig. 18) but had no or little effect on the hexoses. At a concentration of 1000ppm, however, IAA reduced the sucrose concentrations compared to both the control and to 100ppm.IAA, and may thus explain the inhibition of further turion development (see experiment 10, table 20)(page 104)

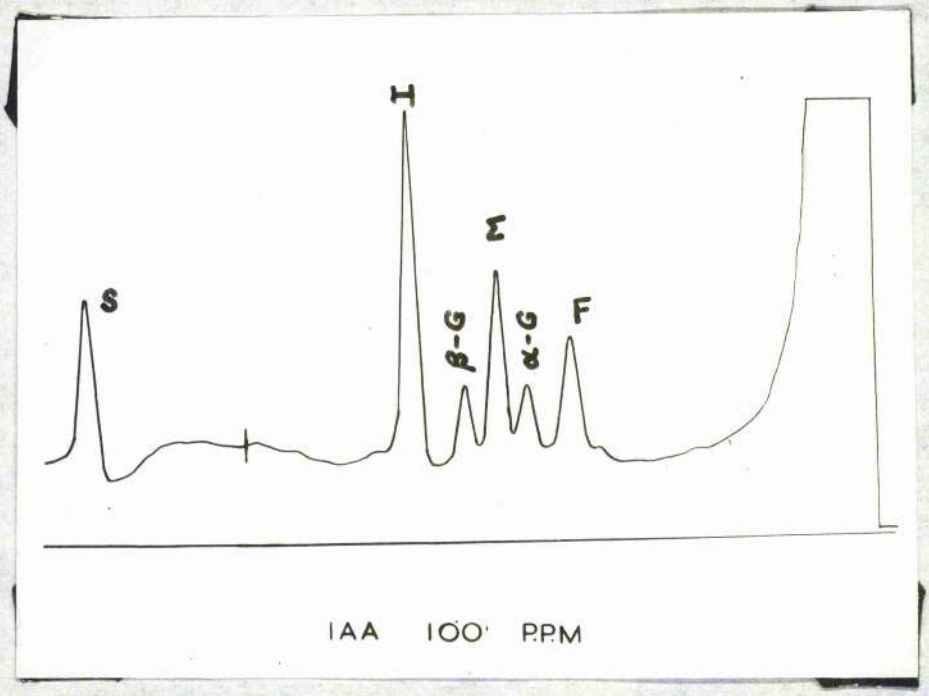


Fig. 18. Soluble carbohydrates of turions exposed to bathing solution of 100ppm. IAA.
Dry wt. turions = 0.0436g. 5ul. attenuation $10x10^2$

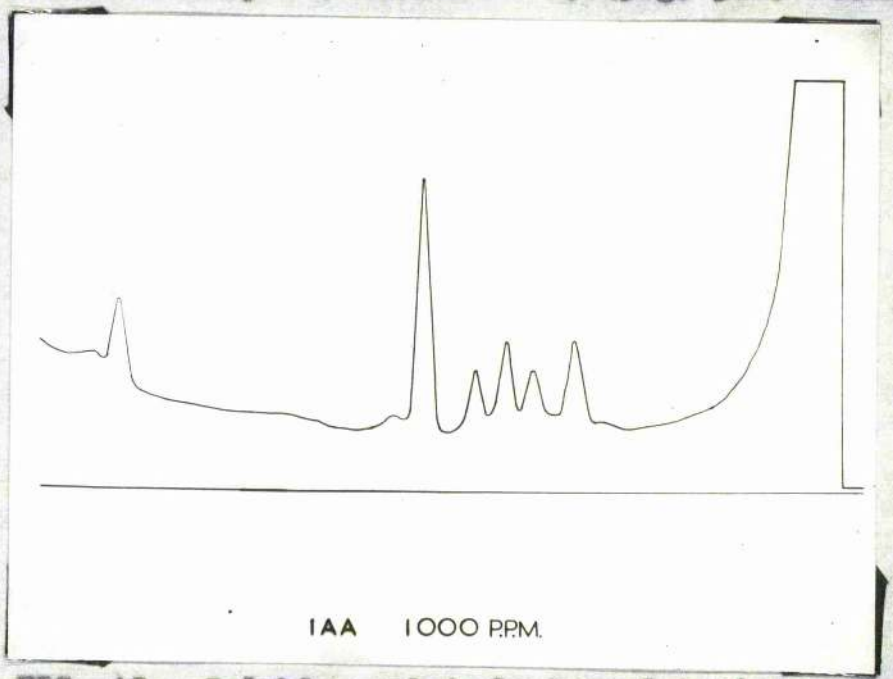


FIG. 19. Soluble carbohydrates of turions exposed to bathing solution of 1000ppm. IAA.
Dry wt. turions = 0.0416g. 5ul. attenuation $10x10^2$.

See page 73 for peak identification.

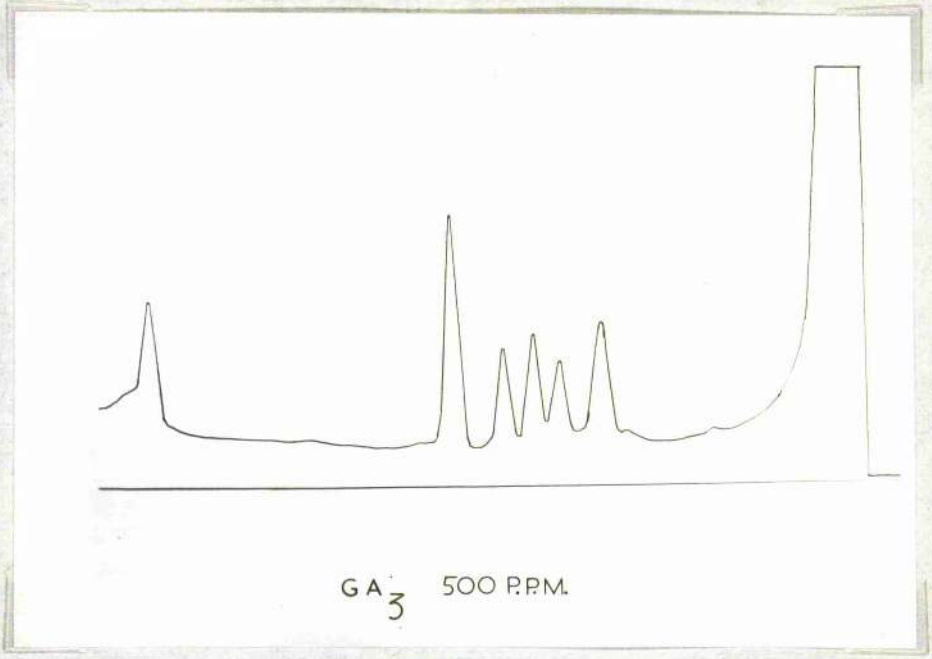


FIG. 20. Soluble carbohydrates in turions exposed to 500ppm. GA_3 for 18hr. Dry wt. turions = 0.0502g. 5µl. injected; attenuation $20x10^2$.

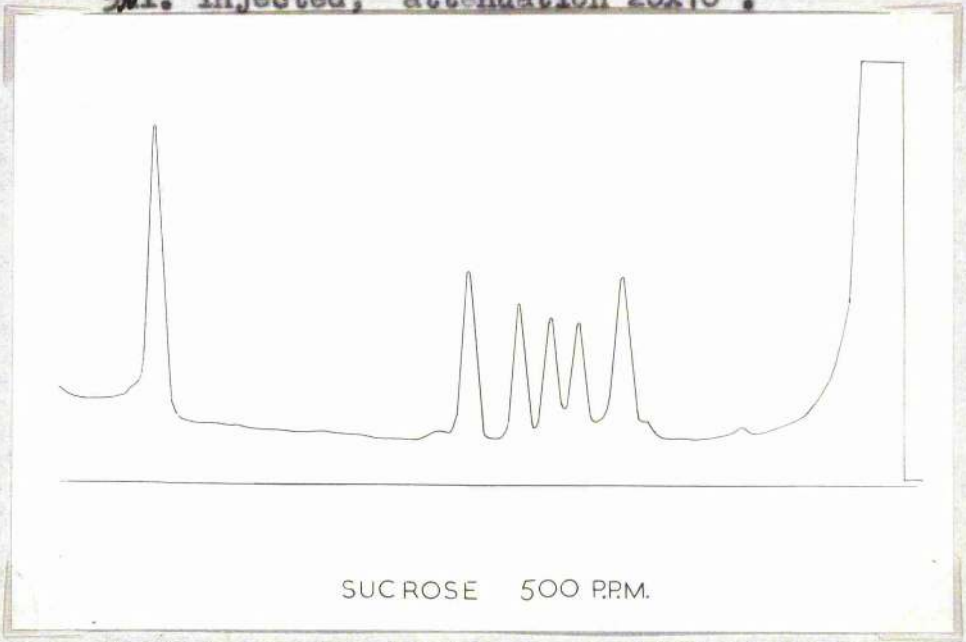


FIG. 21. Soluble carbohydrates in turions exposed to bathing solution of 500ppm. sucrose for 18hr. Dry wt. turions = 0.0500g. 5µl. injected; attenuation $20x10^2$.

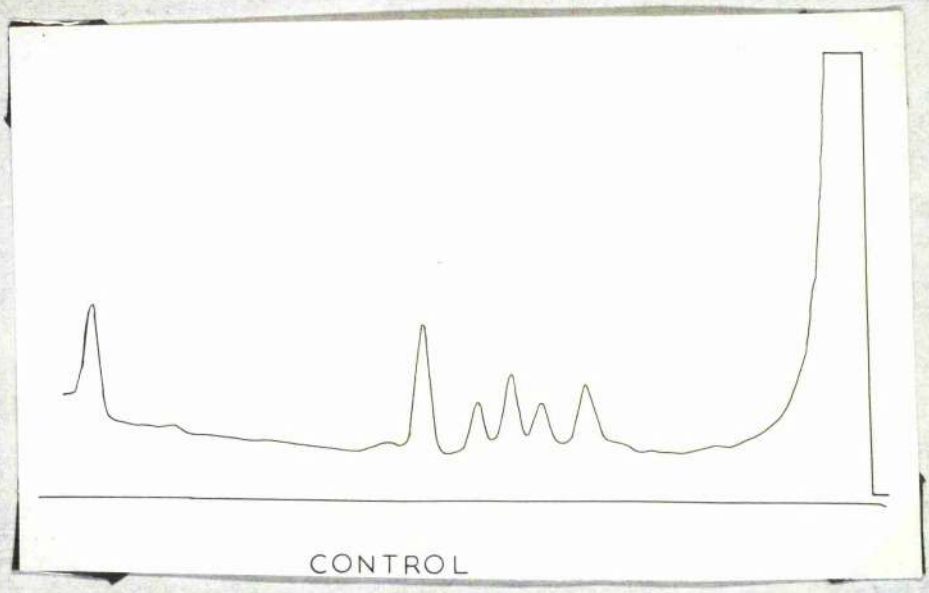


FIG. 22. Soluble carbohydrates in control turions.
Dry wt. turions = 0.0488g. 5ul. injected;
attenuation $20x10^2$.
Controls were held in water.

Were the hormonal acceleration of growth a result of stimulation of sucrose production, one might expect a more rapid response to supplied sucrose than to hormone. This was not found to be so in experiment 5 where it was concluded that the sucrose had a slower rate of penetration than the hormones, and thus hormonal control by increasing sucrose levels cannot be ruled out. Figure 21, page 92 illustrates that supply of sucrose increased the concentration of all carbohydrates. The increase in the hexoses may have been due to microbial breakdown of the sucrose before penetration of the tissue.

Thus it would appear that, for this initial geotropic response, the main change to occur in the carbohydrate regime is an increase in the sucrose levels, and it is a marked increase. The above experiment, where exogenous solutions of hormones and sucrose were used, suggest that IAA levels may be more closely linked with sucrose levels, whilst GA3 may be more closely linked with the hexose levels. Furthermore the fact that the carbohydrate regimes are altered in different ways in response to the two compounds, suggests that the stimulation of growth by exogenous GA3 is not the result of a stimulation of internal auxin levels. Since the main change at the onset of growth is an increase in the concentration of sucrose, it may be inferred that IAA levels at this time are of primary importance.

The significance of sucrose in promotion of growth of the turions has been emphasised above, and the following two chromatograms are included here, as they indicate the signi-

ficance of sucrose in the growth of two other aquatic species.

The chromatograms illustrated in figure 25 is of the soluble carbohydrates in Chara sp. from Loch Drumore in the winter. The plants remain wintergreen and this may be due to their ability to maintain high sucrose levels in their tissue.

Figure 24 illustrates the chromatographic trace of sugars in P. crispus root, and it can be seen that the main soluble carbohydrate in the root is sucrose. No extracts were made of roots from P. obtusifolius M&K, but it is likely that such a carbohydrate regime exists there also. Supporting the direct or indirect control of root emergence and elongation by sucrose is the finding from experiment 10 that exogenous supply of sucrose stimulated both root emergence and elongation (see page 104)

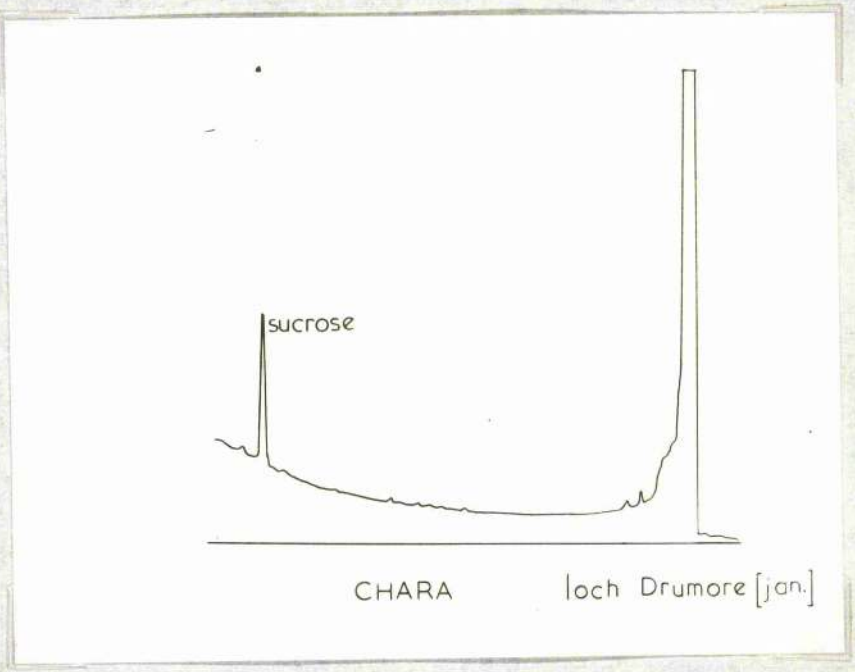


FIG. 23. GLC trace of TMS derivatives of ethanol extract from Chara sp. Fresh wt. tissue = 2µl. injected; attenuation 1 x 10^3.

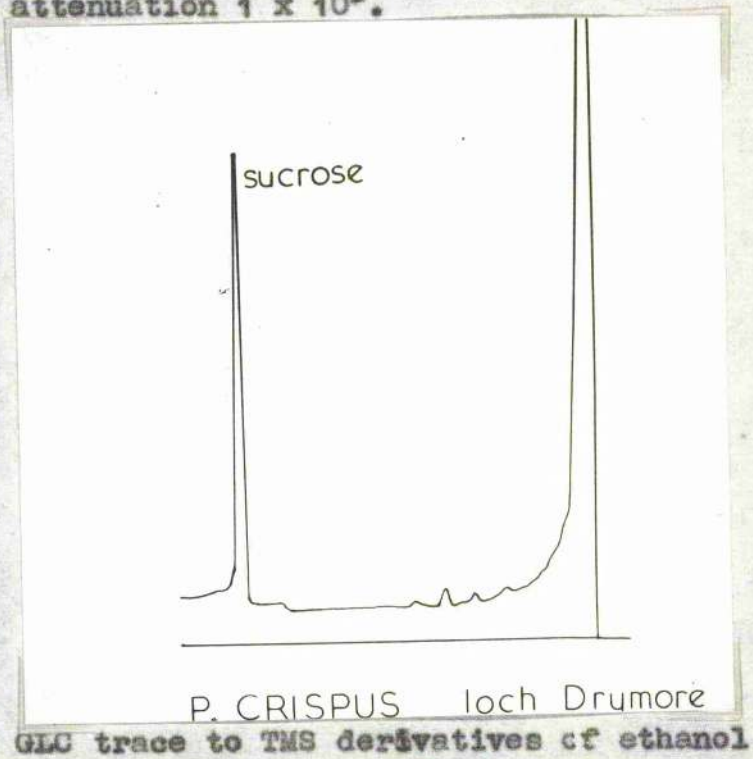


FIG. 24. GLC trace to TMS derivatives of ethanol extract from root of P. crispus Dry wt. tissue = 0.015g. 4µl. injected; attenuation 2 x 10^3.

Geotropism and Soluble carbohydrates : Summary

1. Since both IAA and Gibberellic acid have been tentatively shown to be present in the turions and since both, when exogenously supplied at 5°C, accelerate the geotropic response, it may be concluded that both are involved as agents of the response.

2. Only high auxin concentrations enhanced the response, suggesting perhaps an IAA-induced, ethylene-mediated, response.

3. Turions in the loch during winter, and thus at temperatures about 5°C exhibit no geotropic response. This would indicate that the winter levels of auxin and gibberellins in the turions (or at the site required in the turions) are low.

4. Since ethylene, auxin and gibberellins are known to occur in aquatic species, and since in terrestrial species, all three have been implicated in geotropic responses, they may all be involved in the geotropic responses of the turions. Auxin may bring about the observed increase in plasticity of the stem in the region of 'bending' allowing the GA3-ethylene induced extension growth, demonstrated by Musgrave et al (1972) in Callitriche platycarpa.

5. A study of the soluble carbohydrate levels in the turions of P. obtusifolius MK was carried out using GLC analysis of ethanol extracts from the turions.

6. The turions were found to contain fructose, α, and

β-glucose, mannitol, and m-inositol in large quantities, and sucrose. No maltose was detected.

7. A purification procedure was developed for extracts from a related species, P. crinums, and it was demonstrated that, after purification, the same main peaks appeared, which were neutral and thus carbohydrates.

8. In turions overwintering naturally and in turions held under controlled conditions, the main change in the carbohydrate regime at the time of the geotropic response, and for several weeks after growth initiation, was a dramatic rise in the sucrose concentration. Sucrose, when exogenously supplied was shown in experiment 5 to accelerate the geotropic response, and is thus involved in that response.

9. The carbohydrate changes occurring in turions exposed for 18hr. to bathing solutions of sucrose, gibberellic acid, and IAA, were studied. IAA is probably the main hormonal agent in the direct control of sucrose levels, whilst GA3 is probably more intimately concerned with the control of the hexoses.

10. Turion parts were analysed before and immediately after the initial growth response. The principal carbohydrate in dormant stems was m-inositol, and in the stems of active turions, sucrose. In the leaf, the principal carbohydrate by weight in the dormant and in the active turions was sucrose. In the ligules of the dormant and the active turions the principal carbohydrate was D-glucose. Ligule tissue represented only $\frac{1}{3}$ of dry wt. of the turions, and as the total soluble carbohydrate concentration in the ligules

on a g. sugar/g. dry wt. tissue compared to that for the leaf and stem was not high, it is postulated that the main function of the ligules is perhaps rather to control the gaseous regime of the turions, and so control growth. The starch reserves in the closely-packed parenchyma cells of the basal parts of the turions are probably adequate for the development of the turions.

11. Meso-Inositol was tentatively identified, occurring in high concentrations compared to the normal findings in terrestrial plants; of hormonal levels, that is, about 10^{-7}M. This may be of considerable significance for the rapid growth of the turions in the early Spring.

12. The ability of a species to overwinter as the intact plant may be related to the ability of these plants to maintain high sucrose levels. Chara sp. which was found to contain mostly sucrose in January remains green throughout the winter in Loch Drumore, near Glenshee.

13. The main soluble carbohydrate in the root of P.crispus is sucrose and this may likewise be the case for the roots of the related species P. obtusifolius M&K. This supposition is supported by the promotion of root emergence and elongation by exogenous sucrose, found in experiment 10.

CHAPTER 4.

HORMONAL CONTROL OF STEM, LEAF, AND ROOT GROWTH OF TURIONS

CHAPTER 4 CONTENTS PAGE

A series of experiments follow in this chapter which study the hormonal control of growth of the turions after the geotropic growth response has occurred. The experiments thus consider aspects of growth such as leaf production and elongation, stem growth and root development. Work to date concerning what is known of the hormonal control of stem, leaf and root growth of aquatic plants is presented in a collated form within the body of experiment 10.

EXPERIMENT 10

AIM: To determine whether sucrose, IAA, and Gibberellic acid affect the development of the turions of P. obtusifolius M&K.

MATERIALS: Turions were collected from the Lake of Menteith on December 9th, 1973. The following bathing solutions were used: Sucrose 100, 500, and 1000ppm; IAA 100, 500 and 1000ppm.; GA3 100, 500 and 1000ppm. by weight. Controls were held in distilled water.

METHOD: Twelve turions were held in 250ml. of each of the above bathing solutions for 18 hr. with a 12 hr. daylength at a temperature of 20°C. After this period the turions were washed in distilled water. Several turions per treatment were added to 80% ethanol for subsequent carbohydrate analysis. The growth containers used were beakers which contained a constant amount of John Innes Potting Compost No 1. Six turions were grown per treatment, with three turions being planted per beaker. Illumination was provided by three four-foot 40 watt gro-lux tubes suspended 40cm. above the water level in the beakers. The turions were illuminated for 12hr. per day. The plants were grown for three weeks (14.12.72 - 5.1.73), for by this time differences between treatments were apparent. At the end of this period, some turions were kept as specimens, whilst the others were scored for various growth parameters.

RESULTS: These are presented in table 20, page 104. Representative plants from each treatment are illustrated in plates 25-8.

1. Stem extension was promoted greatly by gibberellic acid, IAA, and sucrose, the greatest promotion with the

gibberellic acid.

2. Leaf production was stimulated by gibberellic acid, may have been stimulated by sucrose, and was inhibited by high IAA concentrations.

3. Leaf elongation was promoted by gibberellic acid, inhibited by high IAA and unaffected by sucrose.

4. Root primordia emergence and elongation was stimulated by gibberellic acid, IAA, and sucrose, in that order of effectiveness.

5. Flowering only occurred in the plants treated with gibberellic acid.

TREATMENT PPM.	NO. LEAVES	STEM LENGTH	NO. ROOTS	LONGEST ROOT LENGTH	LONGEST LEAF
Sucrose					
100	9	2	1	6	4
	10	1	-	-	4
500	12	4	1	6	9
	13	2	-	-	4.5
1000	14	1.5	1	6	6.5
	10	0.5	-	-	2.0
Gibberellic acid					
100	14	14	4	7	8.0
	14	7	2	9	5.5
500	12	5	2	13	4.5
	13	14	2	10	6.0
1000	19	15	3	7	8.5
	14	8	1	2	8.0
IAA					
100	13	3.5	1	8	4.5
	12	2.0	1	9	6.0
500	10	5.0	2	7	3.5
	-				
1000	6	-	2	8	2.5
	6	-	2	2	3.0
Controls	12	0.5	-	-	6.0
	11	1.5	-	-	5.0

TABLE 20. 18hr. exposure period of Effects of exogenous solutions of Sucrose, Gibberellic acid and Indole-3-acetic acid on the development of the turions of P. obtusifolius M&K

PLATE 25. Development of turions in response to IAA

PLATE 26. Development of turions in response to Gibberellic acid.

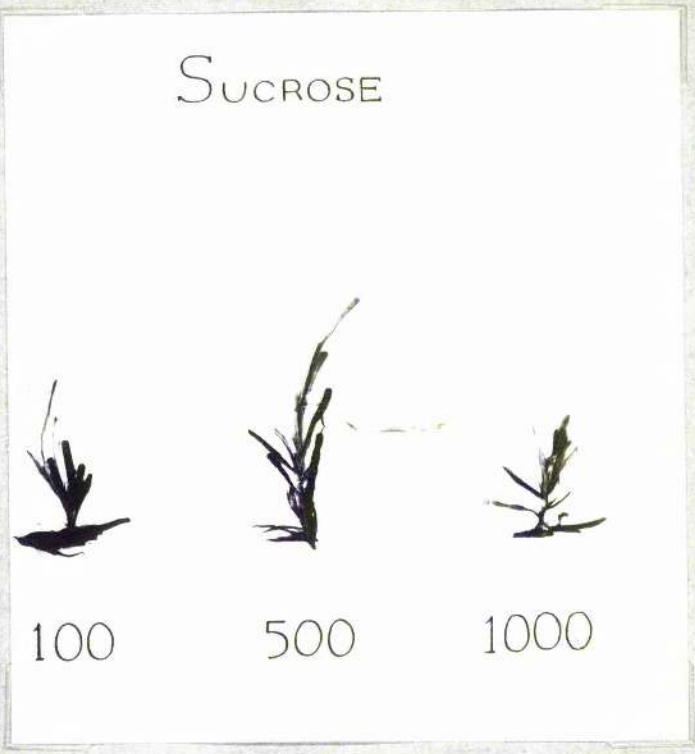


PLATE 27. Development of turions in response to sucrose.(ppm.)

PLATE 28. Development of turions under control conditions.

LITERATURE

Stem growth of aquatic plants (a) Gibberellic acid.

The experimental work to date concerned with gibberellic acid and stem growth of aquatic plants is as follows. In 1961 Brygin et al demonstrated that application of gibberellic acid to seedlings of rice doubled the endogenous auxin. In 1970 Ku et al reported that coleoptile growth of Oryza sativa was stimulated by ethylene, a result which came as a sharp contrast to the reported situation with most terrestrial plants. They also found that instead of competitively inhibiting the action of ethylene, evolved or added carbon dioxide enhanced the response to ethylene. Suge et al (1971) reported the stimulation of oat and rice mesocotyl growth by ethylene. They also found carbon dioxide enhancement of growth, and furthermore a synergism in the action of gibberellic acid and ethylene. Again in sharp contrast to reported work on terrestrial plants, Kyoshi (1972) reported an abscissic acid stimulation of rice mesocotyl growth. The abscissic acid may however have been broken down into the precursor of gibberellins, mevalonic acid. In a later paper Kiyoshi (1973) demonstrated a synergism in the action of abscissic acid and gibberellic acid.

The response of Callitriche stagnalis to gibberellic acid was studied by McComb (1965). On finding that gibberellic acid stimulated the elongation of floating rosettes he suggested that the observed increased rate of growth was due to increase in endogenous gibberellins on submergence of the rosettes. He also noted that floating shoots of the plant

grew more rapidly if 'transpiration' was prevented by smearing the leaves with vaseline. This point was taken up more recently by Musgrave et al (1972) with the related species Callitriche platycarpa. They found that 'optimal' levels of GA3 did not enhance the elongation of the stems to the same extent as did submergence. Submergence allows the accumulation of ethylene. With AMO 1618 pretreatment of the tissue, the shoots did not elongate in response to ETHREL (2-chloroethyl phosphonic acid) and so gibberellin synthesis was shown to be a pre-requisite for ethylene-induced growth.

The latent periods for responses to GA3 and ethylene were 8hr. and 30 minutes respectively, and thus they concluded that ethylene was not active directly through the mode of action of gibberellic acid. Ethylene-enhanced transport of auxin in Ranunculus sceleratus petioles was reported by Musgrave & Walters (1973). Thus the precise, accepted auxin-ethylene balance or feed-back inhibition in terrestrial plants cannot apply here. These workers have also extended their work to Hydrocharis morsus - ranae and Regnillidium diphyllum and ethylene has been found to stimulate the elongation of the petioles and rachi respectively. Finally, Bonnet (1972) reported that the stem of Potamogeton densus was a rhythmical structure, in that it had alternating short and long internodes. Only the longer internodes of the stem could be induced to elongate with gibberellic acid treatment. Here therefore is further confirmation of the differential hormone responses in different growing zones of the same organ as reported more recently by Goto & Esashi (1974).

Discussion: 1. Stem growth - gibberellic acid and sucrose. Experiment 2 (pages 31-3) in chapter 2 demonstrates that under the light regime of the experiment there is an inverse relationship between the length of the period of irradiation and stem extension of the turions. Supply of IAA, gibberellic acid or sucrose overcame the light inhibition to varying extents. Supply of gibberellic acid overcame the inhibition to a much greater extent than did supply of auxin, suggesting perhaps that gibberellins were somehow more limiting under the experimental conditions of growth. Since the effects of these two compounds are so radically different, it is unlikely that gibberellic acid increased endogenous auxin levels, as has often been reported, e.g. by Kuraishi & Muir (1963). Numerous examples of such stimulation are listed in the review paper by Brian (1966), but he points out that auxin never stimulates stem extension to the same extent as does gibberellic acid. The inhibitory effect of the light may have been mediated through one or more of the following mechanisms:

(1) Destruction of endogenous gibberellins.

(2) Inhibition of gibberellin synthesis.

(3) Light alteration in the sensitivity of the tissue to endogenous levels of gibberellins.

(4) Inhibition of ethylene synthesis.

Comparison with similar reported systems indicates that the inhibition may be a combination of (3) and (4). The above situation is similar to that found with dwarf peas. The peas are however only dwarf if grown in the light Lockhart (1956). He demonstrated that the red-light induced inhibition of stem extension could be reversed by GA3 treatment. This was also demonstrated in the normal variety of Alaska, (Phaseolus

vulgaris) which also elongates more rapidly in the dark. Lockhart (1958) demonstrated GA3 reversal of red-light inhibition of stem extension in many species. Work by Kende and Lang (1964) suggested that red light made the tissue less responsive to the endogenous levels of gibberellins. They extracted equal amounts of gibberellins from light and dark grown plants of P.sativum var. Alaska. Two fractions of gibberellins 1 & 11, were isolated chromatographically, fraction 1 being similar in properties to GA5 and fraction 11 similar to GA1. Fraction 1 was highly active in the dark but in light-grown plants GA1 was ten times more active than the inefficient GA5. Fraction 1 could thus be effectively utilised as a growth hormone only by etiolated (normally dwarf) peas. Their data did not suggest conversion of 1 to 11 but rather the existence of two independent hormonal compounds. Such a mechanism of growth inhibition may occur in the turions. There is also some evidence for accumulation of light induced inhibitors. Kohler and Lang (1963) reported the isolation of substances interfering with the response of dwarf peas to gibberellins, since the isolates were tested against GA3 and found to be antagonistic. The speculative nature of much of the work with gibberellic acid is, according to Jones (1973), due to the scarcity of data on the rates of turnover of gibberellin in response to different conditions. Differences in rates of degradation and synthesis may occur and yet give net equal amounts of extractable gibberellins (Zeevart 1971). Gibberellin synthesis and activity we have seen varies greatly in response to red light. Yet red light has been reported to mimic the effects

of gibberellic acid in causing elongation, (Nahamura et al 1966). Reid et al (1968) demonstrated red light induction of gibberellins in barley leaf discs, and reports of growth promoting effects of red light have been made (Mertz et al 1973) which are independent of the response to gibberellic acid. The comparison of work with the turions with the data from the work on dwarf peas however seems a legitimate one as both turions and dwarf peas are characterised by red light inhibition of growth, which can be reversed by gibberellic acid. Furthermore apart from reversal of red light inhibition of growth, GA3 reversal of green and blue light inhibition of growth has also been reported in Alaska pea seedlings (Vlitos et al 1957). Gibberellic acid in this system can thus reverse the inhibitory effect of light over the whole spectrum.

Since there are no reports of ethylene inhibition of growth of aquatic plants, comparison with reports where ethylene has stimulated growth in land plants seems in order, especially since a GA3-ethylene control of growth has been demonstrated in aquatic species. Where ethylene inhibited growth in the plumule of Pisum sativum (Goeschl & Pratt 1967) red light caused a reduction in ethylene synthesis, as determined by its evolution, and in bean and cocklebur hypocotyls prolonged red irradiation caused a reduction in ethylene synthesis, and ethylene stimulated growth (Goto & Esahi 1974). Some reports of ethylene inhibition of extension growth may be experimental artifacts, rather than representative of endogenous control, for Goto & Esahi (1974) found differential responses in different growing zones, and

the length of the period of exposure to ethylene had a great effect on the response. If the exposure time was short then ethylene stimulation of the rapidly growing hypocotyls occurred. Longer periods of exposure blocked elongation growth, and caused radial expansion of the issue, the classical effects of ethylene, as reported for example by Pratt & Goeschl (1967) in P. sativum. Furthermore, Goto & Esahi (1974) demonstrated a GA3-induced increase in ethylene production which occurred in plants grown under red light, whereas auxin enhancement of ethylene was unaffected by the light. This contrasts with the findings of Musgrave et al (1973) in Callitriche platycarpa., that the concentrations of gibberellic acid which caused growth did not stimulate ethylene production. Auxin levels may thus control ethylene production in aquatic plants. In vitro photochemical production of ethylene by a non-enzymic system has been reported by Yang et al (1966) and thus the ethylene may not have been as low in the turions as might have been expected from auxin-induced levels.

Gibberellic acid is reported to induce the formation of a-amylase and also invertase in lettuce (Scott & Leopold 1967). Chromatograms of carbohydrates present in the turions taken from GA3 bathing solutions indicate a slight increase in sucrose and especially in the hexoses (see page 89). Addition of sucrose likewise increased the sucrose and hexose levels. Thus gibberellic acid may control much of the carbohydrate metabolism in the turions. Sucrose concentration rises markedly at the onset of growth of the turions when these are growing under natural conditions. Little work has been done

on the relation between carbohydrate metabolism and stem extension. Frank (1966) found that supply of sucrose to the dormant winter buds of Potamogeton nodosus induced growth. Soni (1972) reported that in rice the peak of invertase activity occurred after 12hr. and this was coincident with the maximum growth rate and these processes had similar kinetics. Addition of sucrose caused an increase in invertase activity, and GA3 and sucrose were 'synergistic' in this respect. He concluded that gibberellic acid and sucrose together may regulate invertase levels. A similar correlation between growth and invertase activity is found in the root of Zea mays (Helleburst & Forward 1962). The apical meristem may require a certain level of sucrose for development as does the root meristem. Weston & Street (1968) report that the sucrose levels established in the root apex appears to be critical to the development activity of the root meristem. This however is probably an effect rather than a cause of the GA3-induced growth. Hatch & Glaziou (1963) found a direct correlation between acid-invertase levels and internode elongation in sugar cane.

LITERATURE AND DISCUSSION

Stem growth of aquatic plants (b) Auxin

Little work has been done on the role of auxin in the control of stem extension in aquatic species. Using an agar block technique, Nomes et al (1937) showed that diffusates from Elodea canandensis promoted internodal axial growth. King (1943) found no stimulation or inhibition of stem growth of Eloden densa at exogenous concentrations of 10-30ppm. IAA.

In 1954 Yamada, studying the growth of rice coleoptile sections in response to IAA, found that the submerged sections were more sensitive to IAA than the floating sections. Seen in the light of recent work on ethylene, this may be explicable on the basis of ethylene accumulation in the submerged sections. In 1960 Inaac found with Elodea canadensis and Najas minor, exposed to auxin solutions for 21 days, and then cultured for 12 months, that IAA promoted stem growth, 'optimal' elongation occurring with 10^{-5}M IAA in each case.

In Callitriche platycarpa (Musgrave et al 1972) the rapid elongation due to submergence, ethylene or gibberellic acid treatment was solely a result of cell elongation, as the undeveloped internodes had their full complement of cells. Both auxin and m-inositol have been demonstrated however to be present in the stem tissue of the turions, and so cell division may also be involved in the extension growth of the stem of the turions, as both affect the mitotic cycles of plants (Davidson & Webster, 1967). Furthermore the observed increase in the plasticity of the stem tissue mentioned in chapter 3 suggests the involvement of auxin in stem extension. Comparison of cell number in undeveloped and expanded internodes would have defined the mode of extension growth of the turions.

If the response of the stems to auxin is induced by ethylene, then the smaller response, compared to that with supplied GA3, may be explained either by a low endogenous GA3 level or by reduced sensitivity of the tissue to gibberellins present. Davies (1972) who studied the fate of exogenously supplied IAA in light grown stems, found that it was rapidly

degraded into a number of metabolites, and he detected no free IAA in the tissue. The finding that exogenously supplied gibberellins can retain their integrity for a longer period of time once inside the tissue, may partially explain their normally greater stimulation of stem extension.

LITERATURE - Hormonal control of leaf growth.

Knowledge concerning the hormonal control of leaf growth in both terrestrial and aquatic plants, is still very scanty. Application of gibberellins to leaves can stimulate both vein and mesophyll growth (Phillips 1971). The auxin and gibberellin contents of leaves are found to be positively correlated with their growth rates. Both gibberellins and cytokinins can stimulate the growth of mesophyll tissue, but depending on the concentration, auxins may stimulate vein growth. Wheeler (1960) reported that in Phaseolus vulgaris the maximum gibberellin content of the leaves coincided with the most vigorous leaf expansion phase. In a review paper Brian (1966) reported that exogenously supplied gibberellins may reduce leaf size in some species, but usually causes an increase in leaf size in grasses. Humphries (1958) observed that the GA3-induced increase in leaf growth may not always persist, for he found that with Phaseolus vulgaris the final areas of control and treated primary leaves were the same. Gaudet (1968) found that there was a steep rise in soluble sugars at the expense of starch, in the youngest leaves of Marilea vestita at the water depths, where the very elongated leaves were being produced. This suggests the involvement of gibberellic acid, as it controls, perhaps synergistically with ethylene the degradation of starch reserves, into hexose

sugars. Ethylene has been reported to inhibit leaf expansion (Abeles 1973).

Cytokinins appear to be the most important factors controlling chlorophyll retention in the leaves. The need for a root system to maintain the aerial parts may be replaced by a source of cytokinins, and further Kuleava (1962) has demonstrated cytokinin activity in the root exudates of sunflower. Leaves treated with gibberellic acid normally have less chlorophyll than controls (Bishop et al 1961). Recently Yakushkina (1972) reported that gibberellin treatment of corn chloroplasts decreased the retention of the chlorophyll, whilst kinetin increased the retention.

DISCUSSION: Leaf growth of turions.

Sucrose may have shown some slight stimulatory effect on leaf elongation and leaf production (see table 29, page 104) Gibberellic acid increased both leaf number and also stimulated the elongation of the leaves. Since IAA treatment did not overcome the inhibitory effects of the light with respect to leaf growth, it is unlikely that low IAA levels are responsible for lack of growth in the controls. If the effect of supplied IAA was to boost ethylene production then growth of the leaves would still have been inhibited by the postulated low gibberellin levels, as gibberellin synthesis has been reported above to be a pre-requisite for ethylene induced elongation. IAA may also have had an inhibitory effect on the photosynthesis of the leaves, as reported by Dickson et al. (1963) working with Lemna. At the higher concentration of IAA used with the turions however, there may

have been some tissue damage. In Callitriche platycarpa (Musgrave et al 1972) the linear-leaf form could be produced only by gibberellic acid treatment. Much of the leaf growth of the turions consists of increase in length and it is thus likely that gibberellic acid exerts considerable control over this phase of development.

LITERATURE: Hormonal control of root growth of aquatic plants.

Work concerned with root growth in aquatic plants has been focussed dn the effects of exogenous solutions of natural and synthetic auxins on root emergence, and development. Such work was carried out by Soltys et al (1938), King (1943) and Inanc (1960), with species of Egeria, Elodea, and Najas. King (1943) found that IAA promoted root hair formation stimulated the emergence of the dormant root primordia from the cortical tissues, increased the average root length, and increased the number of roots of Elodea densa. Similarly Inanc (1960) found that a 10^{-8}M solution of IAA proved to be 'optimal' in promoting root initiation, and development, in Elodea canadensis and Najas minor. This effect was enhanced by artificial aeration of the cultures, suggesting perhaps enhancement of elongation by carbon dioxide, as found in the stem growth of rice. Musgrave et al (1972) found that with Callitriche platycarpa both ethylene and submergence completely inhibited the appearance of new adventitious roots. There appears to be no other reports of any effects of gibberellic acid on root growth in aquatic plants. Dale (1957) showed that with Elodea canadensis roots grown in the light were

morphologically different from dark-grown roots. The equivalent of dark-grown root could be produced in the light if ethylene was bubbled through the culture solutions. Abeles (1973) has reported that ethylene may promote the emergence of pre-formed root primordia.

DISCUSSION: Root growth of turions (a) Gibberellic acid.

(c) Gibberellic acid stimulated both the emergence of root primordia and elongation of roots (see table 20). Wider ranges of concentrations of each compound would have to be used before the relative roles of gibberellic acid and IAA could be assessed. Furthermore the shorter roots induced by IAA cpd. to those induced by gibberellic acid may only reflect a slower growth rate. Gasparikova (1972) reported that a decrease in the growth rate of roots in response to IAA solutions (10^{-4}M -- 10^{-7}M) was due to a shortening of the period of cell elongation. This however was on a much shorter time scale. In most plants root development involves radial growth of cells, then longitudinal extension. The role of gibberellins in root growth of terrestrial plants is only now being considered. There is good evidence that gibberellins are synthesised by the roots of some plants (Jones & Phillips 1966, Lang 1970, El Antably et al 1974). That the response to gibberellic acid is an 'independent' one is suggested by the reports of Odnoff (1963) and Lacoppe & Gaspar (1968) who found that gibberellins have little effect on the auxins in roots. Gibberellic acid has been reported to both stimulate root growth, and inhibit it. As far as root initiation is concerned gibberellic acid application is found

to be markedly inhibitory. Tognoni et al (1967) found that supply of GA3 caused inhibition of root growth, whilst Devlin and Brown (1969) found that gibberellins promoted root development. A GA3-ethylene mechanism for cell elongation in the roots of the turions may thus exist, with IAA controlling the level of ethylene and regulating cell division with cytokinins.

DISCUSSION: Root growth of turions

(b) Sucrose.

(a) Exogenously supplied sucrose stimulated the emergence of the root primordia in the turions and also promoted root elongation (see table 20).

Weston and Street (1968) reported that sucrose levels established in root apices appeared to be critical with respect to the size and activity of meristems. This indication that carbohydrates are closely involved in the formation of root primordia is supported by the work of Stoutenmyer and Britt (1962), who found that supplying sucrose to the 'difficult-to-root' Hedera canariensis increased its ability to root. The sucrose supplied to the turions may have stimulated invertase activity and so increased hexose levels, or may be used directly by the roots. There is a close relationship between invertase activity and cell elongation in the roots of Zea mays. Helleburst & Forward (1962) found that over the period of cell elongation in the roots, there is a forty-fold increase in invertase activity. The effect of supply of sucrose on the carbohydrate regime of the turions

is discussed in chapter 3, experiment 9. The experimentally 'evolved' media used in root culture work, such as agar, with IAA, cytokinin, m-inositol, and sucrose indicate the principal factors involved in root growth. With regard to root meristem culture, Christiansen and Thimann (1950) reported a preferential use of sucrose, with no parallel loss of reducing sugars. The specificity of sucrose, is also stressed by Wetmore and Rier (1963) and Jeffs and Northcote (1967) in work concerned with vascular strand initiation in callus cultures in response to applied auxin and sugar.

(b) IAA, likewise promoted the emergence of root primordia and the development of the roots in the turions of P. obtusifolius M&K. Street (1966) has shown that root growth does not occur in plants in the absence of auxin while Shapiro (1958) reported that formation or growth of root primordia in forest trees may be inhibited by light, especially if the light reaches the area in which they are developing or normally would develop. Lack of growth of the root primordia of the turions in the controls may thus be a result of such a mechanism. Auxin supplied to plants may conjugate with aspartic acid in intact roots and root segments, (Scott 1972). Both IAA and Inositol have been found in the turions, and as mentioned above both have been shown to affect mitosis in plants, and to be required for division. In tomato Sinha (1968) found that the addition of meso-inositol to the culture medium prolonged the linear growth of the roots and the functioning of the cambium. Further, a tentative isolation of an IAA - inositol complex has been made by Nicholls

(1967), and such a complex may mediate auxin-induced cell division in roots. As lower concentrations of IAA were not tested root development may have been a result of ethylene induced by IAA. Emergence of pre-formed root primordia in response to ethylene has been reported by Abeles (1973). King (1943) however, found that lower concentrations of IAA promoted emergence of root primordia (see above). Krishnamoorworthy (1970) reported that IAA and ethylene, as ETHREL, were synergistic in promoting root number and root elongation in mung bean hypocotyl. Roots of many aquatic plants including those of P. obtusifolius have lacunae, and a synergistic mechanism such as this may therefore be involved in the control of root growth. In terms of emergence of the root primordia and their development root growth is later shown to be inhibited by the experimental light regime, if the light reaches the area where the primordia would normally emerge. Thus a lack of auxin in the region of the root primordia through destruction of auxin or through inhibition of transport may inhibit their development. If auxin is present in the leaves and is not transported to the roots then no root development can occur. Although the literature is conflicting on the effect of light on longitudinal auxin transport, a report on light and basipetal transport of auxin by Maqvi & Gordon (1967) with IAA - ^{14}C applied to corn seedlings which were illuminated bilaterally with white light, indicates that light causes a significant decrease in the amount of transported IAA. Greenwood (1970) studying root regeneration in Pinus lambertians embryos found that TIBA treatment, which "specifically" inhibits auxin

transport, prevented rooting, but supply of IAA promoted root formation. Since cytokinins are involved in cell division in most plants they probably play a joint role with the other major hormones. They may however have an inhibitory role, since Krishnamoorworthy (1970) found that kinetin inhibited rooting in mung bean.

FLOWERING: Only plants treated with gibberellic acid flowered under the experimental conditions. Thus as the plants flower in the loch during mid August-early September, gibberellic acid may replace the requirement for long days in the experiment. Immediately prior to flowering in the loch the growth of the plant is characterised by the rapid elongation of the internodes, and leaves. Both high temperatures and long days are known to increase the turnover of endogenous gibberellins.

EXPERIMENT 11

AIM: This experiment was designed to determine what concentration of exogenously supplied gibberellic acid was required to affect the development of the turions.

MATERIALS: Turions were collected from the Lake in January 1974.

METHOD: Turions were visually matched and then tied singly and carefully to short lengths of glass rods with nylon thread, as there is a reduction in the S.G. of the turions at the onset of growth. The weighted turions were inserted into 250ml. measuring cylinders containing 250ml. distilled water for the controls, or the same volume of gibberellic acid in concentrations between 10^{-12}M and 10^{-6}M. The measuring cylinders were placed in constant temperature baths at 15°C. Three three foot 40 watt gro-lux tubes suspended 15cm. above the tops of the measuring cylinders illuminated the plants for 12 hr. per day. This daylength was chosen as most of the stem extension in nature occurs during the early summer where the daylength is about 12 hr. The turions were scored for various growth parameters after 3 weeks, as differences between the plants from different treatments were apparent.

RESULTS: Table 21 presents the MEANS± S.E. for the growth parameters of turions from the various bathing solutions. The student's 't' test was used to test the significance of the means difference of growth parameters of the turions from the gibberellic acid solutions compared to the controls. Significant means differences for the values of the growth parameters compared to the controls were found only with turions from the 10^{-6}M GA3 bathing solution. The dramatic change in the response of the turions to supplied GA3 at this concentration is seen in plate 29. The significance of the means difference between the controls

and turions from 10^{-6}M/litre GA3 bathing solution are presented in table 21 in asterisked row ***

MOLARITY GA3	INTERNODES	STEM LENGTH	LEAF NO.	ROOT NO.	ROOT LENGTH
NIL (4)	12.5±0.43	2.94±0.08	11.5±0.56	0.25±0.21	0.07±0.06
10^{-12}(4)	11.25±0.65	2.51±0.45	9.5 ±0.56	0.5±0.25	0.2±0.14
10^{-10}(5)	9.4±0.80	2.11±0.09	8.2±0.66	0.2±0.18	0.08±0.07
10^{-8}(5)	10.4±0.67	2.70±0.24	8.6±0.54	0.4±0.22	0.04±0.02
10^{-6}(4)	13.25±0.65	4.50±0.07	9.75±0.65	2.75±0.65	5.12±2.75
***	(NS)	(P 0.01)	(NS)	(P 0.01)	(NS)

TABLE 21. Turion development in response to various concentrations of GA3.

The promotion of internode extension by gibberellic acid was measured and the results are presented in table 22.

LENGTH INTERNODE (cm.) GA_3 10^{-6}M (4)	CONTROLS (4)	INTERNODE NO. (from base of turion)
0.20±0	0.21±0.01	1
0.25±0.01	0.22±0.01	2
0.27±0.03	0.27±0.01	3
0.35±0.01	0.34±0.01	4
0.42±0.02	0.31±0.01	5*
0.55±0.04	0.39±0.03	6*
0.50±0.04	0.31±0.01	7*
0.55±0.05	0.24±0.03	8*

TABLE 22. Stimulation of internode extension of turions by 10^{-6}M solution of gibberellic acid.

* Asterisked cases show GA3 stimulation of internode extension.

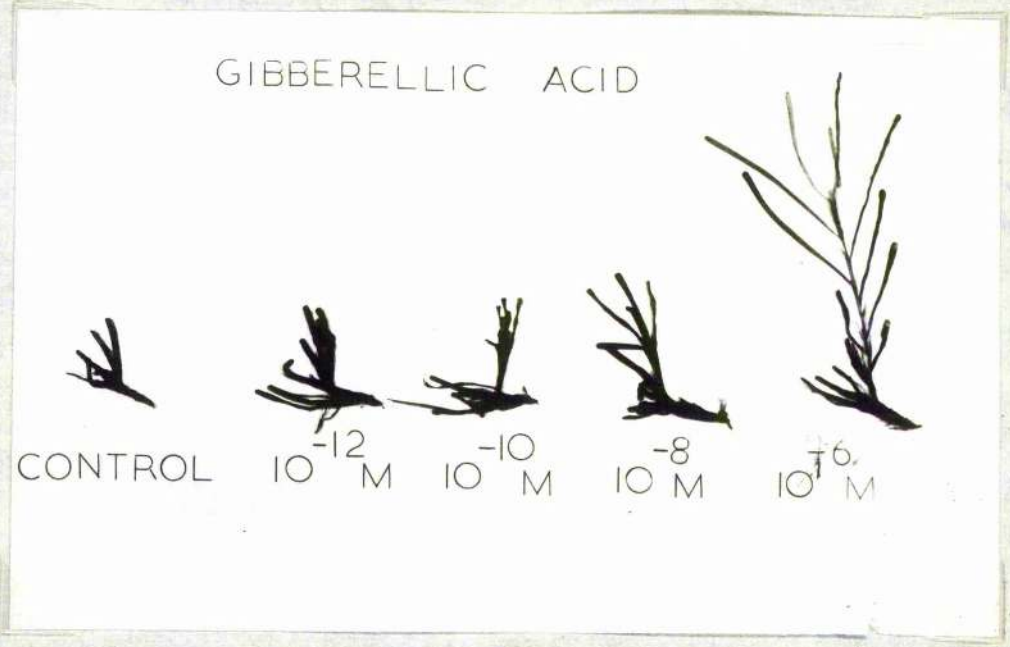


PLATE 29. Turion development in response to various concentrations of exogenous gibberellic acid.

The promotion of growth of only certain internodes of the stem tissue of the turions is interesting. The four lowest internodes in most turions represented the part of the stem which consisted of closely packed parenchyma cells containing starch, and these did not extend by treatment of the turions with GA3. The part of the stem above this zone of storage tissue possessed lacunae, which thus may allow diffusion of ethylene to sites of growth, whereas lack of lacunae in the more basal parts of the turions would prevent or restrict the GA3-ethylene postulated growth of the turions.

CONCLUSION: Under the given experimental conditions only 10^{-6}M/litre GA3 had a significant effect on the development of the turions. This concentration of exogenous GA3 is used in the following experiment where the control of temperature over promotion of growth by supplied GA3 is studied.

Figure 2 page [18] illustrates that most of the stem growth of the turions occurs during the early summer, when the temperatu of the lake was about 15°C, and when the plants are growing under long photoperiods. It was considered relevant to study the growth of the turions in response to gibberellic acid at different temperatures, for there have been several reports of temperature dependent GA3 stimulation of growth in terrestrial plants. Cline et al (1970) proposed on the basis of his findings, a high-temperature control mechanism to operate for gibberellin production in the mountain rosette form of Achillea. Adams et al found that the promotion of elongation of Avena stem segments by gibberellic acid which was 15 times the control elongation was temperature dependent. Guttridge (1961) reported a temperature dependence in the promotion of leaf area growth in Fragaria by gibberellic acid. The following experiment determines whether such a temperature dependence exists for GA3 stimulation for growth of the turions.

EXPERIMENT 12

AIM: To investigate the promotion of growth of the turions by gibberellic acid, at different temperature.

MATERIALS: Turions were collected from the Lake of Menteith on January 9th, 1974. The gibberellic acid bathing solution used was 10^{-6}M., as the previous experiment showed this to be a suitable concentration.

METHOD: Turions were selected for the experiment if they were 4cm. in length from the base of the stem to the leaf apices. They were tied to short lengths of ghass rod to hold them submerged throughout the experiment. 250ml. measuring cylinders were used as growing containers. Five control turions in distilled water, and five turions each held in 250ml. gibberellic acid were grown at 5, 10, 15 and 20°C, and in each case 14hr. illumination was provided by three gro-lux tubes. The lighting arrangement was as in experiment 11. Likewise the duration of the experiment was 3 weeks (15.2.74-7.3.74).

RESULTS: Plates 30-3, pages 129-130, illustrate the responses of the turions at each temperature, with and without gibberellic acid. The results for the various growth parameters are presented in histogram form in figures 25-8, pages 131-2. Table 23 lists the significance of the means differences for these parameters from plants from the different treatments. Striped histograms represent the MEANS±S.E. for parameters for turions treated with gibberellic acid. The effect of temperature was analysed statistically using analysis of variance, and the findings are shows an table 23.

1. Leaf growth. Temperature had a significant effect on leaf elongation, both in the controls and in turions treated with GA3. In neither treatment did temperature have an effect on leaf production.

2. Stem growth. Temperature affected internode production in control turions and more so in GA3-treated turions, and a similar effect was found for stem elongation.

3. Root growth. Only at temperatures of 15°C and 20°C was there any significant temperature stimulation of root emergence and elongation, and this only occurred with the GA3-treated turions.

	Leaf length	Leaf no.	Internodes	Stem length	Root length	Root no.
TEMP. EFFECT						
Controls	P<0.001	(NS)	P<0.05	P<0.05	(NS)	(NS)
GA	P<0.001	(NS)	P<0.001	P<0.001	P<0.005	P<0.005
GA3 EFFECT						
5°C	(NS)	(NS)	(NS)	(NS)	(NS)	(NS)
10°C	(NS)	(NS)	(NS)	(NS)	(NS)	(NS)
15°C	(NS)	(NS)	(NS)	P<0.01	P<0.05	P<0.05
20°C	P<0.01	(NS)	P<0.05	P<0.01	(NS)	P<0.01

TABLE 23. Significances of differences between means. **for growth parameters, for the effect of temperature and the effect of GA3 at different temperatures.**

The effect of gibberellic acid on development was analysed using the 't' test on the means, and the results are as shown in table 23 (lower block), and in the histograms in figures 25-28.

1. Leaf growth. Only at 20°C had gibberellic acid any

significant effect on leaf elongation, but it had no effect on leaf production at any temperature.

2. Stem growth. Gibberellic acid had an effect on internode production only at 20°C and promoted stem extension at 15°C and 20°C.

3. Root growth. Gibberellic acid promoted the emergence of root primordia and the elongation of the roots at 15°C and 20°C.

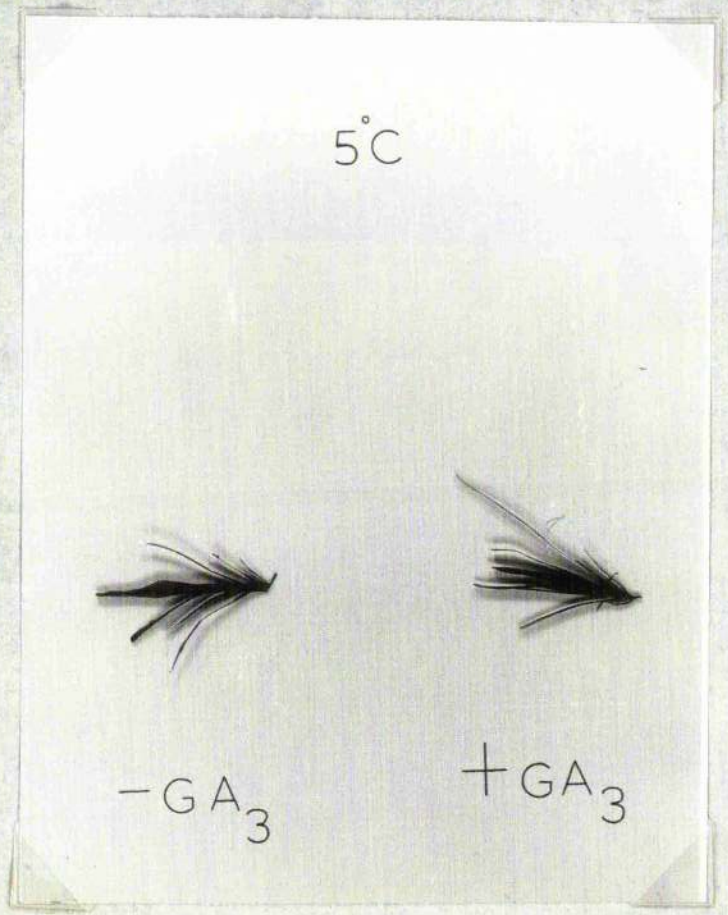


PLATE 30. Response of turions to GA3 at 5°C

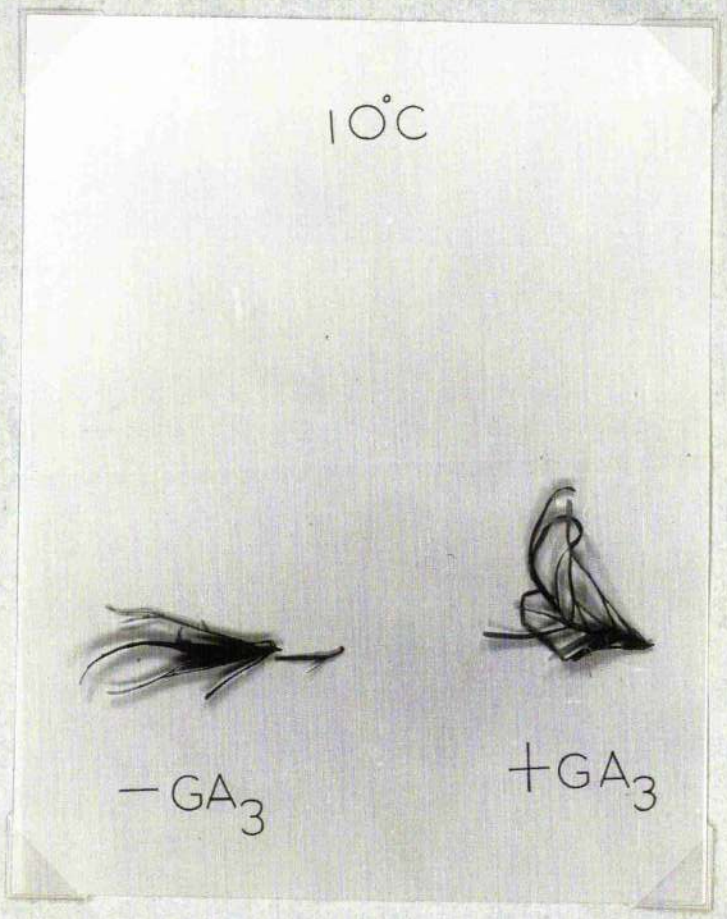


PLATE 31 Response of turions to GA3 at 10°C.

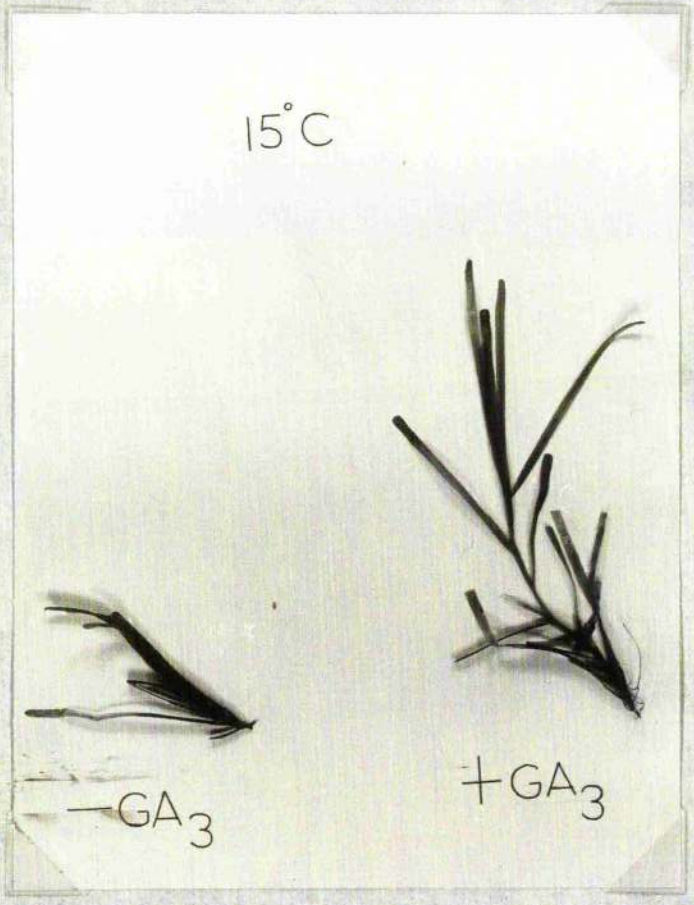


PLATE 32. Response of turions to GA3 at 15°C

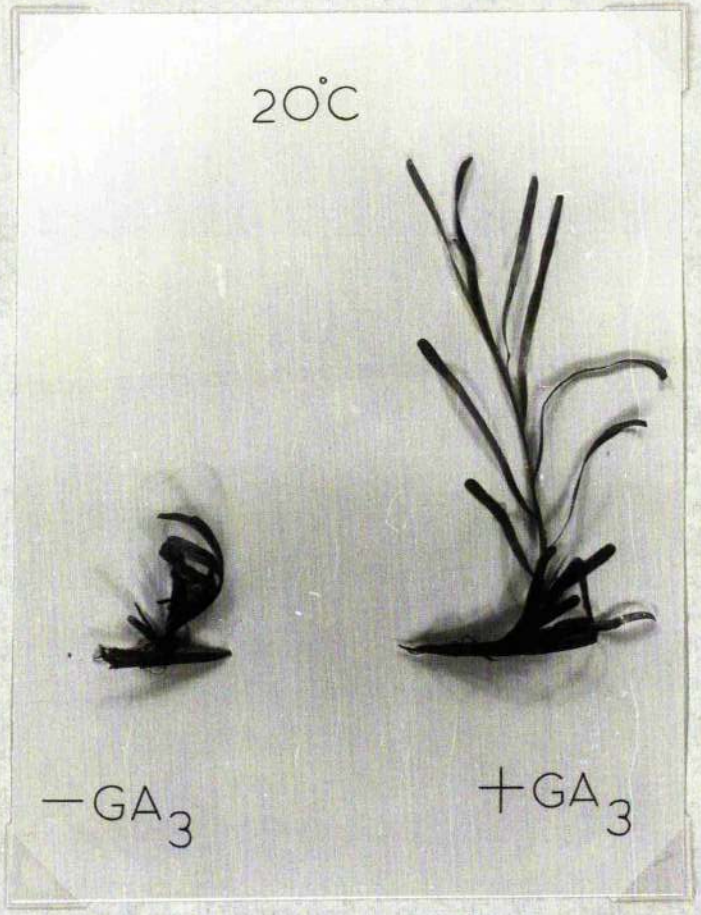


PLATE 33. Response of turions to GA3 at 20°C

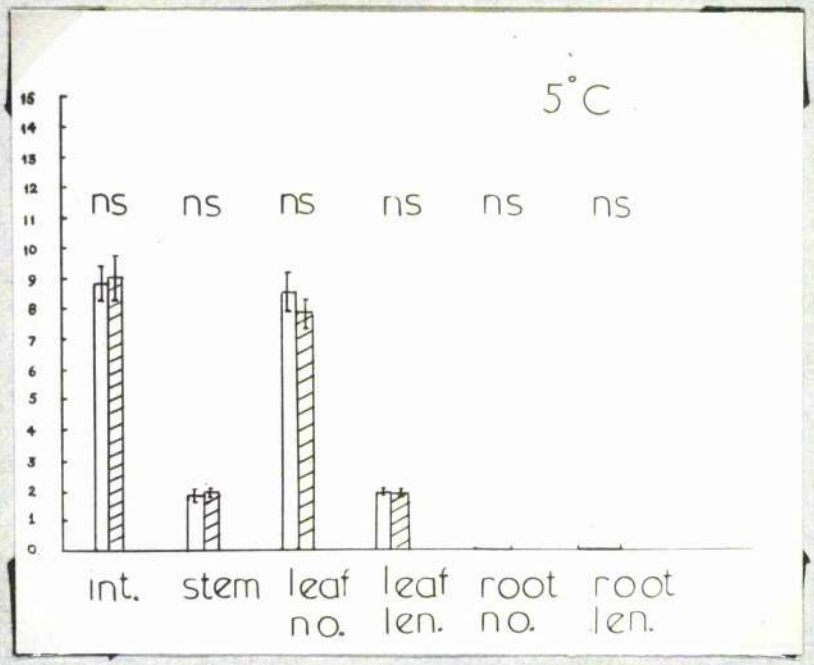


FIG. 25. Response of turions to 10^{-6}M GA3 at 5°C. Results presented as MEAN±S.E. in figs. 25-8. Striped histograms represent values for parameters for turions exposed to GA3.

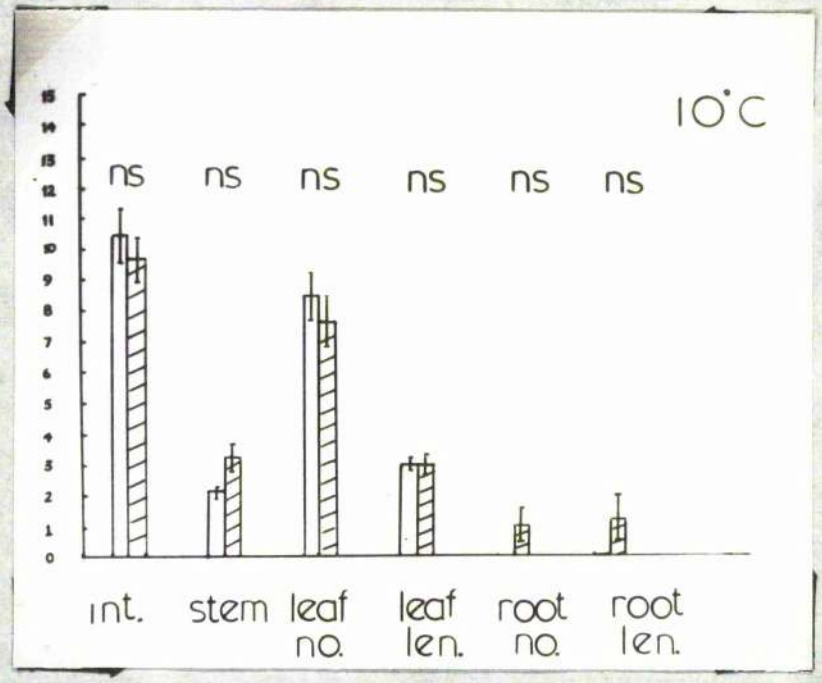


FIG. 26. Response of turions to 10^{-6}M GA3 at 10°C.

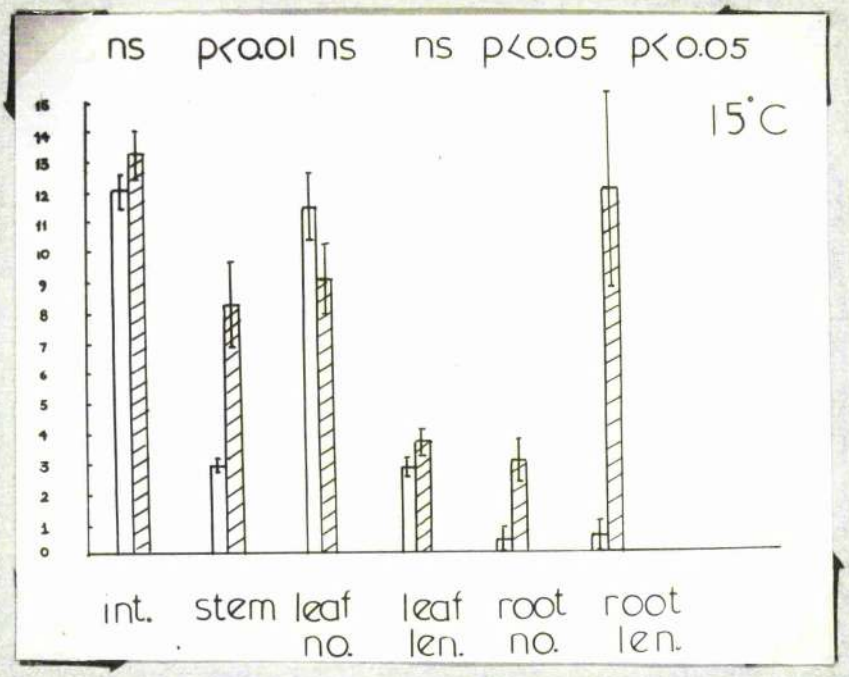


FIG. 27. Response of turions to 10^{-6}M GA3 at 15°C

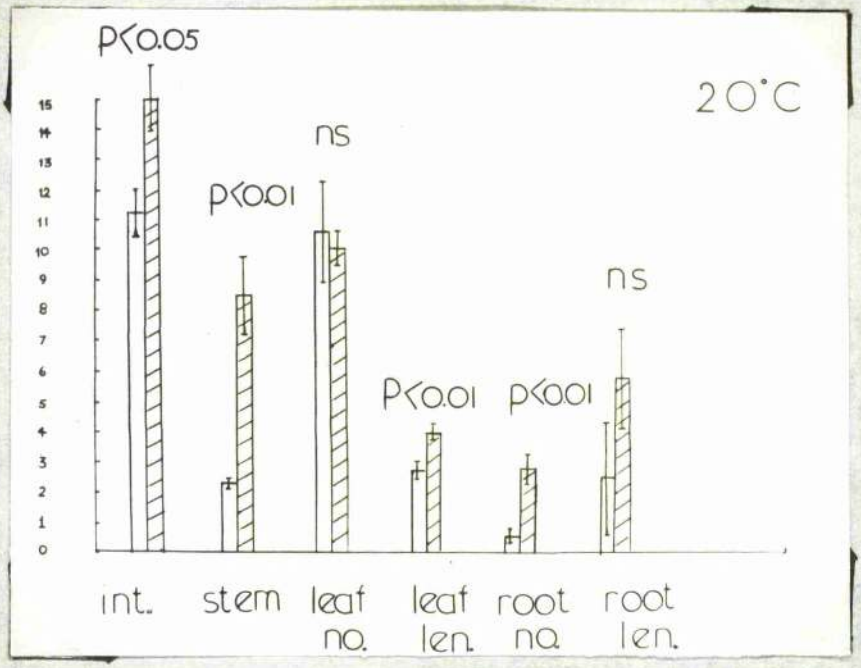


FIG. 28. Response of turions to 10^{-6}M GA3 at 20°C

DISCUSSION:

1. STEM GROWTH

There was significant promotion of stem elongation by gibberellic acid only at 15°C and 20°C. From plate 31 it appears that there is a slight effect at 10°C. There is certainly a dramatic change in the response of the turions to the supplied gibberellic acid at 15°C (see plates 31-2). √√ Temperature significantly effected stem elongation in the controls, but more so with turions treated with gibberellic acid.

Little more can be inferred from the above than that the stem growth of the turions is temperature dependent, and that the response to gibberellic acid is a response of an already active turion. Thismay indicate the importance of other factors controlling stem elongation of the turions, or may be a reflection of the different total gibberellins levels, 'natural' and supplied, present at different temperatures in the turions.

2. ROOT GROWTH

Root growth under natural conditions begins at temperatures between 6-9°C (see fig. 1, page 8). Experiment 2 illustrates in plate 15, page 32, that the emergence and development of the roots depends on supply of some agent from the shoots, because with increasing daylength to which the aerial parts were exposed, there was a decrease in root emergence and elongation. Root growth has been also shown to be partly controlled by the temperature of the water (Expt. 1, page 26, fig. 7). Some temperature stimulation of root growth occurred in this experiment, but much more so in turions treated with gibberellic acid, indicating perhaps that under normal

conditions (where root primordia are not exposed to the light) gibberellic acid may be transported from the shoots to the root primordia, thus allowing rapid development. The root primordia of the turions are surrounded by starch-rich parenchyma cells (see plate 8, page 16), and a possible mode of action of the gibberellic acid may be to stimulate the release of soluble carbohydrates from these cells. Sucrose supplied exogenously has been shown to stimulate root emergence and development. Translocation of such carbohydrates will probably be increased with increasing temperature.

3. LEAF GROWTH

Under the experimental conditions neither temperature nor gibberellic acid stimulated the production of new leaves, suggesting perhaps a light inhibition of this process. Temperature had a significant effect on leaf elongation, both in the controls and in turions treated with gibberellic acid. Only at 20°C was there any stimulation of leaf elongation by gibberellic acid. Plate 15, page 32, illustrates that leaf elongation under the experimental light regime is greater the shorter the period of illumination.

CONCLUSION: Thus from this experiment we have a correlation between the ability of gibberellic acid to stimulate stem, leaf and root growth, and temperature. Similarly it has been shown, and observed in nature that there is a correlation between most facets of growth and the temperature of the water. It does not immediately follow that use of gibberellins partly controls growth of these plants, but since it has been shown that the extension growth of *Callitriche platycarpa* is dependent on an ethylene-gibberellin mechanism of growth, (Musgrave *et al* 1972), then I believe that it is fair to underline the real possibility that gibberellin metabolism plays a significant role in the growth of the turions.

EXPERIMENT 13

AIM: To determine whether ethylene is involved in the stem growth of the turions of P. obtusifolius M&K.

MATERIALS. ETHREL E was obtained from Marks & Co. Ltd.. The nutrient solution used was Hoagland's half strength. The concentration of ETHREL chosen were 0.5mg/litre, 0.5mg/litre, and 5mg./litre. The concentrate supplied contains 48gm. 2-chloroethylphosphonic acid per litre. The concentrations used were those used by Musgrave et al (1972), when studying the elongation of Callitriche platycarpa.

METHOD: Plants were held in the floating or submerged positions in 250ml. bathing solutions, in measuring cylinders, which were placed in constant temperature baths at 15°C. The plants were illuminated for 2hr. per day, for ethylene control of growth is often greater in dark-grown plants or those grown in dim-light. 1 gro-lux tube illuminated the plants laterally, to ensure that all plants received the same amount of light, whether floating or submerged. Plants were scored initially for stem lengths, and rescored after 12 days, when loss of chlorophyll from the leaves was becoming apparent. The experiment was carried out between 29.5.74 - 10.6.74.

RESULTS: Results are presented below in table 24.

TREATMENT		STEM LENGTHS (cm.)					
		FLOATING			SUBMERGED		
		IN.	FINAL	INC.	IN.	FINAL	INC.
CONTROL	1	2.7	2.7	0	2.7	2.7	0
	2	2.7	2.9	0.2	2.2	2.2	0
	3	3.5	3.5	0	2.2	2.3	0.1
ETHREL	1	2.7	2.7	0	3.2	3.2	0
0.05	2	2.0	2.4	0.4	3.0	3.0	0
	3	3.0	3.4	0.4	2.7	3.4	0.7**
0.5	1	2.0	2.9	0.9**	2.7	3.0	0.3
	2	2.8	2.8	0	2.8	3.0	0.2
	3	2.4	2.4	0	3.4	3.7	0.3
5mg/l	1	2.5	2.5	0	3.4	3.5	0.1
	2	2.3	3.0	0.7**	2.4	2.7	0.3
	3	4.4	4.5	0.1	2.7	2.7	0

TABLE 24. Stem elongation of turions, in response to 0.05, 0.5, and 5mg/litre ETHREL, in half-strength Hoagland's solution. [see methods for cultural conditions]

DISCUSSION: The results in table 24 are very variable, and attention here is focussed only on the asterisked cases. The greatest response to ethylene in the submerged plants occurred in 0.05 mg/litre ETHREL, whilst with the floating plants a similar growth response occurred in 0.5mg/litre and 5mg/litre ETHREL. This suggests that ethylene produced in the stem tissue of the floating plants is lost more readily by diffusion into the air. Musgrave et al (1972) reported

a similar situation with Callitriche platycarpa, where accumulation of endogenous ethylene was considered to account for the more rapid growth rates of submerged stems. They also found that the growth rates of the stems of the submerged rosettes could not be stimulated by ETHREL treatment which suggested a saturated system, under submerged conditions. That there was a response to ETHREL in the turions even when the turions were submerged suggests that under the experimental conditions the level of ethylene present was low, or alternatively that the sensitivity of the tissue to endogenous ethylene was low.

POTAMOGETON OBTUSIFOLIUS M&K

SUMMARY

1. The natural life cycle of this species is described in detail as observed in the Lake of Menteith, near Aberfoyle, Perthshire. For a comparison with results with experimental work, a collection of representative plants from each phase of the life cycle was made.

2. The plant overwinters as turions, which are telescoped plants which develop rapidly from early Spring into the mature elongate plants.

3. The evidence presented in the thesis does not categorically define whether the winter rest period is true dormancy, or whether it is simply an environmentally imposed rest period.

4. At the onset of growth the turions may elongate or exhibit a geotropic growth response. The turions were analysed for gibberellins using the agar diffusate technique and lettuce hypocotyl technique, and gibberellins were tentatively identified as being present in the resting turions. The Avena assay used in the detection of auxin activity in the turions was invalidated due to incorrect experimental technique. The geotropic response of the turions was accelerated when high concentrations of gibberellic acid IAA or sucrose were provided in the external solution, suggesting their involvement. The results of Experiment 6 where only high auxin concentrations of 10^{-4}M accelerated the geotropic response suggests the possible involvement of ethylene. Studies of the soluble carbohydrate changes in the turions at the onset of growth in the loch, and experimentally, using GLC analysis of ethanol extracts demonstrated that there was a dramatic seven-fold

increase in the concentration of sucrose over these periods, in the stem of the turions. Since sucrose was shown to accelerate the geotropic response, then it may be inferred that it is involved in this early stage of growth of the turions. Carbohydrates present in the turions exposed to bathing solutions of IAA, GA3 and sucrose for 24hr. were analysed, and IAA appeared to affect the concentration of sucrose whilst GA3 affected the hexose levels. This evidence and the plasticity of the stem tissue at this stage indicate that auxin may play a significant controlling role.

5. The turions were extracted in hot ethanol and the soluble carbohydrates found using GLC analysis over a temperature range of 100-250°C were fructose , and glucoses, manitol, m-Inositol, and sucrose.

6. High concentrations of meso-inositol were found in the turions and this may explain partially the rapidity of the development of the turions.

7. Since one of the major environmental factors regulating growth is temperature the effect of temperature on the growth of the turions was studied. It was found that all facets of growth stem, leaf, and root growth were partially controlled by temperature. The design of the experiments does not permit a discussion of the relative significance of the role of light in such processes.

8. One of the main aspects of growth studied was stem extension. In nature this occurred most rapidly at temperatures between 15-20°C, during the summer months. Long daylengths and high temperatures are known to increase the turnover of gibberellins, in some terrestrial plants (Zeevart 1971) and thus it was considered worthwhile to study the

promotion of growth of the turions by gibberellic acid.

A preliminary experiment with hormonal solutions of gibberellic acid, IAA, and sucrose, demonstrated that gibberellic acid could stimulate the growth of all parts of the turions whilst both IAA, and sucrose stimulated the growth of only certain parts. Further circumstantial evidence in favour of partial control of growth by gibberellins comes from the observation that when 10^{-4}M GA3 was supplied to the turions only then did the plants flower. In nature the plants flower in August when the temperature of the lake is about 20°C, and the daylength is about 14hr.. This flowering of the plants coincides with the period of maximum stem extension. Experiment 12 where the promotion of growth of the turions with gibberellic acid at 5°, 10, 15, and 20°C was studied demonstrated a temperature dependence in the ability to use supplied gibberellic acid. Only when the plants were already active could they utilise it, indicating the importance of other factors in the initiation and the subsequent extension growth.

9. As mentioned in the conclusion to experiment 12, the work brings out two main correlations, growth and temperature, and temperature and the ability of the turions to use supplied gibberellins. The connection between growth and gibberellins, if any is still therefore tenuous, but in the light of the work at Cambridge (Musgrave et al 1972) it seems reasonably fair to propose the connection as real. Some promotion of growth of the turions was noted in response to supplied ETHREL M.

CHAPTER 5.

LIFE CYCLE OF LITTORELLA UNIFLORA (L) ASCHERS

LITTORELLA UNIFLORA (L.) ASCHERS

Life cycle. This is an extremely adjustable plant, as it may grow terrestrially, emergent or submerged. The plants may flower if they are growing terrestrially, or are emergent, but when submerged their sole means of propagation is by stolons. The morphology of the rosettes is simple - compressed stem axis, linear leaves, and roots. In the following study, two aspects of the growth of this species are considered in some detail (a) propagation and (b) leaf elongation. The species was considered particularly worth studying, for it covers vast areas of shorelines of freshwater lochs throughout Britain, with few apparent nutritional demands.

(a) Propagation. When colonising eroded shorelines L. uniflora propagates by means of stolons, and the possible hormonal controls involved in stolon growth are considered in chapter 6. If the rosettes are subjected to competition or are repeatedly covered by sediment, then the rosettes alter their rooting levels to cope with the rising sediment. Arber (1920) first reported the ability of the plants to alter their rooting levels. Previous work of my own (Webster 1971, honours exercise) demonstrated that when the rosettes are partially and repeatedly covered by sediment, the leaves elongate, with roots forming on what is or was leaf tissue (see plate 34, p. 144) If the rate of sedimentation is high enough then a vertical rhizome-like structure is formed, with the various rooting levels reflecting the sequential nature of the deposition (see plate 35, p. 144) Under such conditions there is little stolon

production. Plants illustrating the changes in rooting level under natural conditions were found and collected at Loch Drumore, near Glenshee, in Perthshire. The sward was very dense, and next to the inflow, where there was an obvious fan of silt. These specimens are lodged in the herbarium. Although not described in the following work, rosettes were buried in sand, with an without ETHREL to determine what controlled such vertical perennation, but there was no positive response to the supplied ethylene.

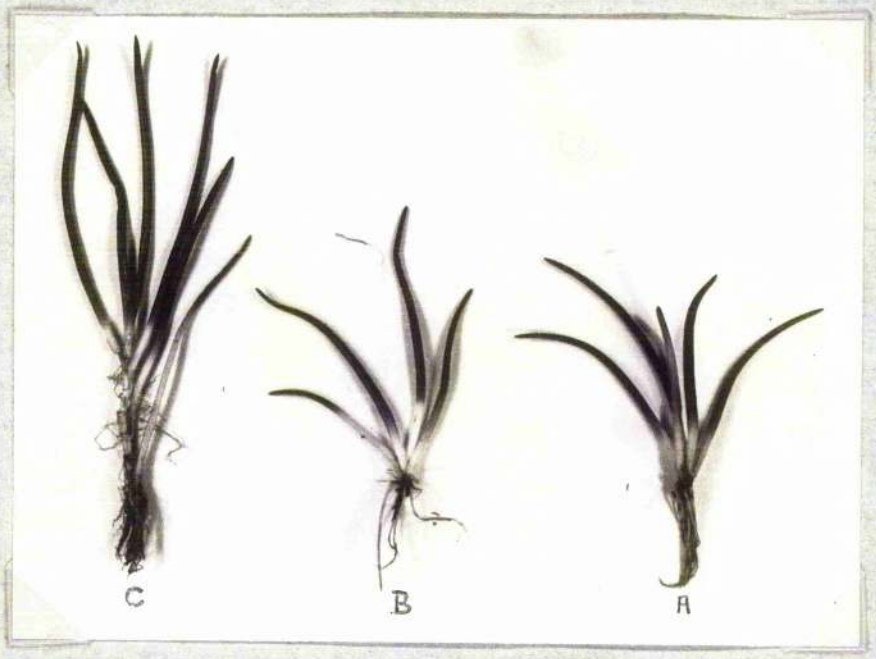


PLATE 34. Effect of increasing rates of deposition on elongation of leaves.

Legend: A - ½cm. per month
B - 1 cm. per month
C - 2cm. per month.

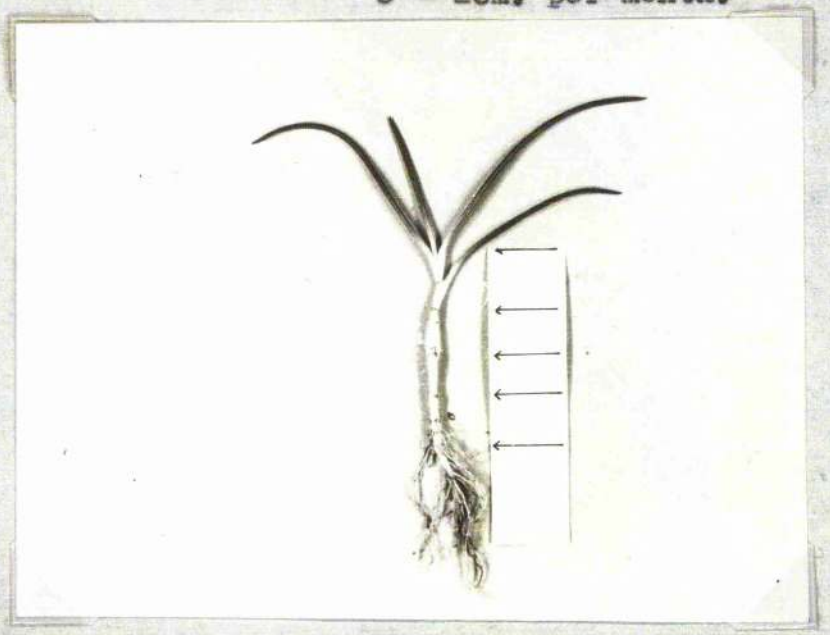


PLATE 35. Production of vertical rhizome-like structure, with rooting levels corresponding to sequences of burial by sediment. (2cm. per month).

(b) Leaf growth. Leaf elongation of this species increases generally with increase in water depth. This was first reported by Arber (1920), but she did not define whether this was due to a changing mud regime or increasing light attenuation or both. Evidence in favour of the mud regime controlling leaf elongation comes from work presented below (Webster 1971, Honours exercise), where it is shown that the mud may strikingly affect the leaf elongation of the rosettes even when the rosettes are growing in shallow water. Deeper water tends to be richer in the silt fraction, and hence richer in cations. An elongation of leaves of the rosettes of this species with increasing depth of water under equal nutrient conditions has not been demonstrated experimentally, but Aberg (1943) demonstrated from field data, that the leaves of a co-habiting rosette species, Lobelia dortmanna, elongated in response to the increasing light attenuation. In his work, the change in the light regime in the different areas of study was the main variant. In the experiment mentioned on the preceding page concerned with rates of sedimentation there was increasing leaf elongation with increasing rates of burial, and this may purely have been an effect of changes in the quantity or quality of the light received by the leaves or leaf meristems. Terrestrial leaves which develop from submerged rosettes which are exposed to air, elongate rapidly, but there is little radial growth of the leaves, and very little lacunal production (see plate 36 page 147). Possible hormonal agents involved in the control of leaf growth of the rosettes are considered in the following work.

With decreasing depth of water rosettes in some lochs become redder in appearance. This pigment, probably

anthocyanin, is present in the epidermal cells of the leaves. This can be seen on the south shore of Loch Brandy in the Cleish Hills, Kinross-shire. This production of anthocyanin may be related to endogenous ethylene levels. Shallow water plants are exposed to more red light and thus lower ethylene levels would be expected. Akamine (1965) reported that the fading of Vanda orchid blossoms involved the peroxidation of anthocyanin pigments. Ethylene is known to enhance peroxidase activity in some tissues (Imaeohi et al 1968). Kang et al found that in etiolated cabbage seedlings stimulation of anthocyanin synthesis by brief exposure to red light was completely prevented by applied 10ppm. ethylene and IAA. Where escape of endogenous ethylene was allowed synthesis of anthocyanin was accelerated. The seedlings produced enough ethylene endogenously to affect anthocyanin production.

Like so many aquatic plants, the storage carbohydrate is starch, which occurs in the leaves (see fig. 29, page 148) and also at the root meristem. Starch formation in the leaves occurs in plastids, two grains being formed per plastid.

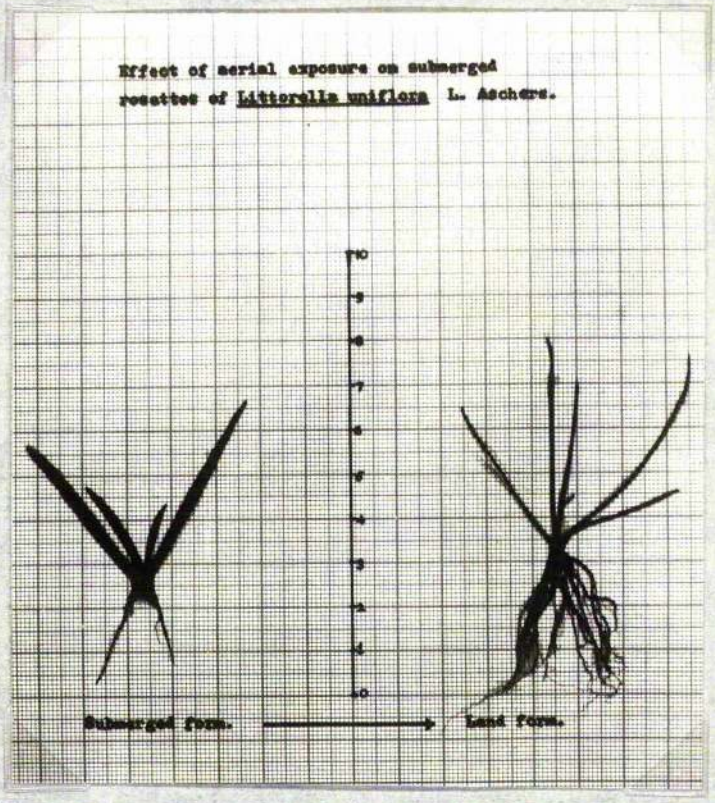
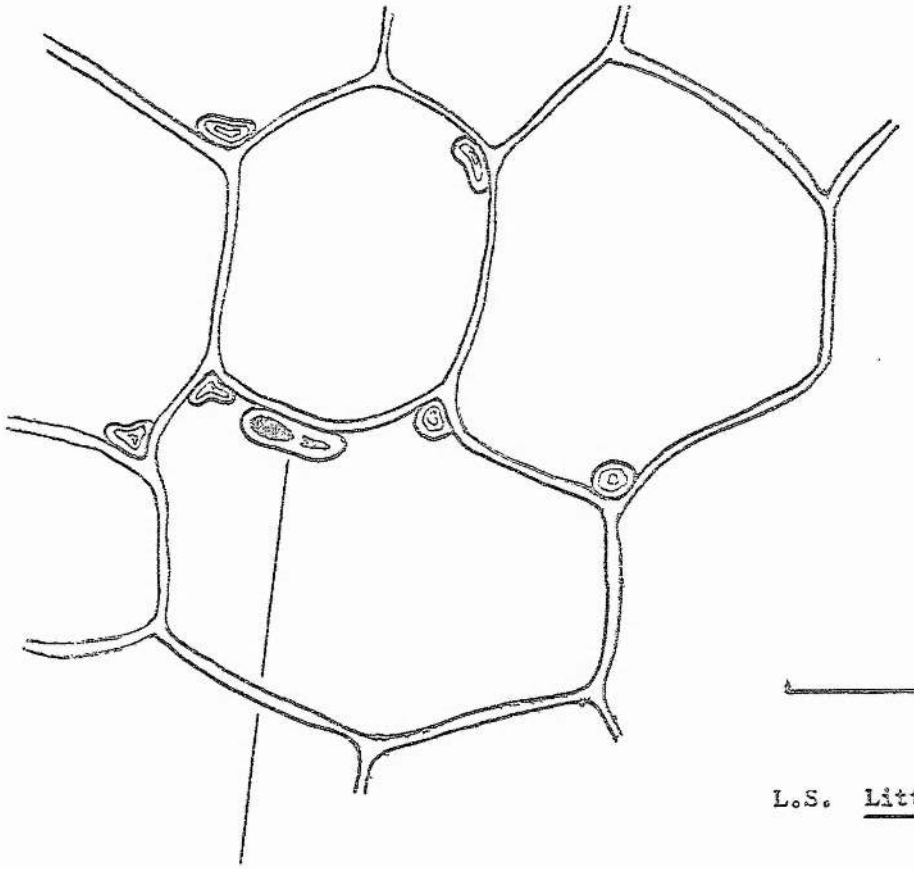


PLATE 36. Effect of aerial exposure on submerged rosettes.

PLATE 37. Stomata in terrestrial rosette leaf tissue.

STARCH FORMATION



x 40

0.1mm.

L.S. Littorella uniflora (L.) Aschers.

Two developing starch grains within plastid.

FIGURE 28 STARCH FORMATION IN LITTORELLA UNIFLORA (L.) ASCHERS

Comparison of terrestrial and submerged forms of L. uniflora.

If the water level is maintained at root level, then the leaves of submerged rosettes slowly senesce, and terrestrial leaves are formed. The induced changes in the morphology of the rosettes are illustrated in plate 36, and listed below in table 25.

LAND	SUBMERGED
Numerous leaves	4 - 6 leaves
Long thin leaves	Squat leaves
Leaves bilaterally symmetrical	Leaves radially symmetrical
Coiled roots	Roots generally straight.
Few lacunae in leaves	Lacunae extremely well developed (see plate 38)
Lateral roots present	Little development of lateral roots
Few lacunae in roots	Lacunae well developed (see plate 39)
Stomata present (see plate 37)	Stomata ± absent

TABLE 25. Comparison of morphology of terrestrial and submerged rosettes.

The roots of submerged rosettes are easily bruised by injury, and permanent damage results from the consequent waterlogging of the lacunar system. In the experiments in this section, the rosettes were handled extremely carefully. Damage to the leaf tissue likewise results in abscission of the tissue.

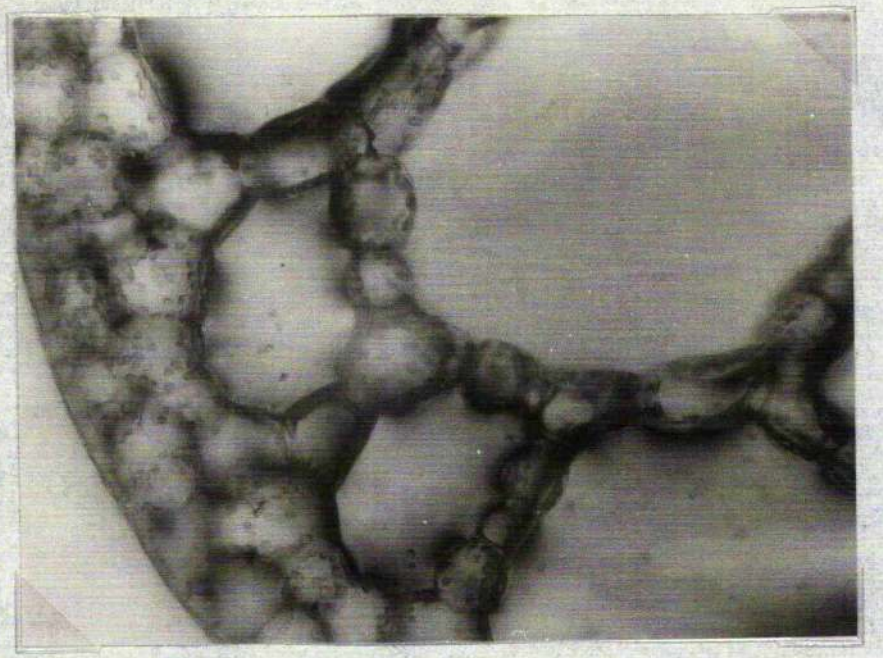


PLATE 38. Lacunal development in leaf of submerged rosette.

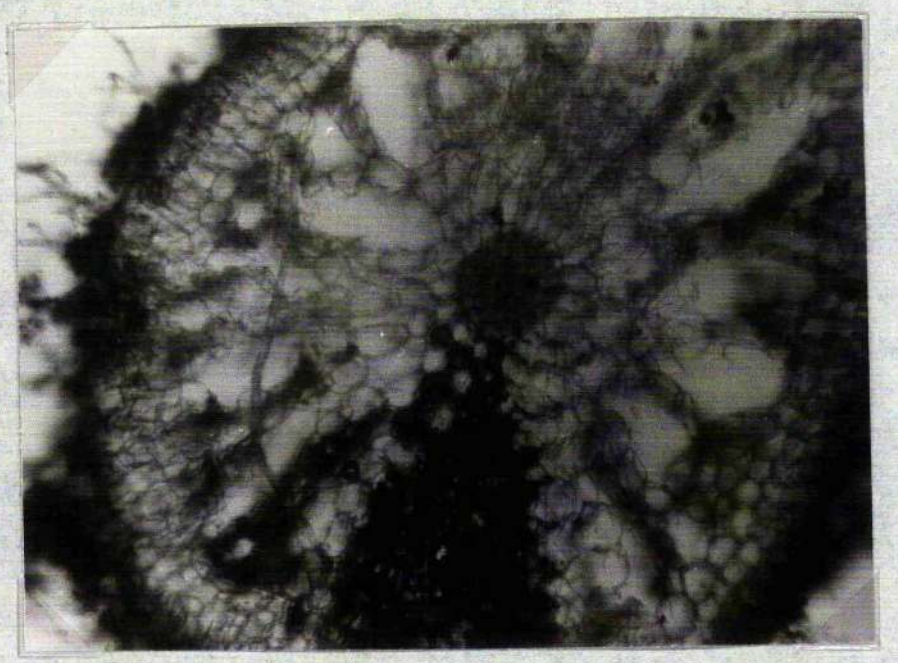


PLATE 39. Lacunal development in root of submerged rosette.

CHAPTER 6.

STOLON GROWTH OF LITTORELLA UNIFLORA

EXPERIMENT 14.

INTRODUCTION: Where completely submerged the rosettes of Littorella uniflora propagate solely by means of stolons. The stolons are diageotropic, and growth remains in the horizontal plane until the leaves of the terminal plantlet are well differentiated, when the direction of growth becomes vertical. Roots are then produced from the developing plantlet. Stolons of varying lengths attaching rosette to rosette may persist for considerable periods of time or may senesce. This experiment studies the elongation of the stolons.

MATERIALS: Rosettes were collected from a shallow bay on the east side of Lindores Loch, nr. Newburgh in Fife, on 16th July, 1974. Rosettes were visually matched and selected for the experiment if they had a short length of stolon which did not have a clearly differentiated terminal plantlet. Rosettes were held in the following bathing solutions for 24hr. in the dark at 20°C:

Gibberellic acid	10^{-6}M, 10^{-4}M.
IAA	10^{-6}M, 10^{-4}M.
ETHREL	0.5mg/litre, 5mg/litre.
CONTROLS	Distilled water.

METHOD: Five rosettes were held in 1 litre of each of the above bathing solutions. On removal from the bathing solutions, the rosettes were washed in distilled water, planted, and held under 10cm. water (approximate growing depth in loch). Illumination was provided by 3 gro-lux tubes (three feet in length, 40 watt each) for 14 hr. period per day. The gro-lux tubes were held 40cm. above the surface of the water in the tray.

Rosettes were scored for stolon length every 2-3 days over the experimental period of 12 days. By this time stolon growth had ceased and differentiation of the terminal plantlets was occurring.

RESULTS: Table 26 indicates the raw data obtained.

STOLON LENGTH (cm.)

	16th	19th	21st	23rd	25th	28th
	1.9	1.9	1.9	1.9	1.9	1.9
	2.1	2.6	2.6	2.7	2.7	2.7
CONTROLS	2.5	3.0	3.8	4.0	4.0	4.0
	2.7	2.7	2.7	2.7	2.7	2.7
	3.0	3.1	3.1	3.1	3.1	3.1
	1.2	1.6	2.0	2.3	2.5	2.5 UD*
	1.5	2.2	2.8	3.2	3.2	3.2 UD*
GA3 10^{-6}M	2.2	2.2	2.2	2.2	2.2	2.2
	2.3	2.5	2.8	2.8	2.8	2.8
	3.2	3.8	3.8	3.8	3.8	3.8
	1.3	2.3	3.3	4.3	4.6	5.7 UD*
	1.8	1.9	2.0	2.2	2.2	2.2
GA3 10^{-4}M	1.9	2.4	2.9	2.9	2.9	2.9
	2.4	2.6	3.0	3.0	3.0	3.0
	2.8	3.3	3.3	3.5	3.5.	3.5
	1.6	1.6	1.6	1.6	1.6	1.6
	1.9	2.2	2.2	2.2	2.2	2.2
IAA 10^{-6}M	2.3	2.6	3.4	3.5	3.6	3.6
	2.6	2.6	2.6	2.6	2.6	2.6
	3.2	3.2	3.2	3.2	3.2	3.3
	2.0	2.4	2.5	2.7	2.7	2.7 UD
	2.3	3.7	4.0	4.0	4.0	4.0
IAA 10^{-4}M	2.7	3.7	3.7	4.2	4.2	4.2
	3.7	3.7	3.9	3.9	3.9	3.9
	0.7	0.9	0.9	0.9	0.9	X
	2.6	2.6	2.6	2.6	2.6	X
ETHREL	3.3	3.3	3.3	3.3	3.3	3.4
0.5mg/litre	3.3	3.3	3.3	3.3	3.3	3.3
	3.5	3.7	3.7	3.7	3.7	X
	1.1	1.3	1.3	1.5	1.5	X
	1.2	1.7	2.0	2.8	2.8	2.8
ETHREL	3.1	3.2	3.3	3.3	X	X
5mg/litre	3.4	3.4	3.4	3.4	3.4	X
	4.1	4.1	4.2	4.1	4.1	X

UD - Undifferentiated terminal plantlet; X - Senescent

TABLE 26. Stolon growth of L. uniflora. in response to a 24 hr. exposure period, in the dark to various growth substances.

STOLON ELONGATION 16th - 19th

INITIAL LENGTH	1-2cm.	2-2.5cm.	2.5-3.0	3.0-
CONTROLS	1.9-1.9(1.9)	2.1-2.6(2.9)	2.5-3.0(4.0) 2.7-2.7(2.7)	3.0-3.1 (3.1)
GA3 10^{-6}M	1.2-1.6(2.5) 1.5-2.2(3.2)	2.2-2.2(2.2) 2.3-2.5(2.8)	---	3.2-3.8 (3.8)
GA3 10^{-4}M	1.3-2.3(5.7) 1.8-1.9(2.2) 1.9-2.4(2.9)	2.4-2.6(3.0)	2.8-3.3(3.5)	---
IAA 10^{-6}M	1.6-1.6(1.6) 1.9-2.2(2.2)	2.3-2.6(3.6)	2.6-2.6(2.6)	3.2-3.2(3.3)
IAA 10^{-4}M	2.0-2.4(2.7)	2.3-3.7(4.2)	2.7-3.7(4.2)	3.7-3.7(3.9)

Figures in ( ) are final lengths of stolons.

TABLE 27. Stolon growth of L. uniflora in three days.

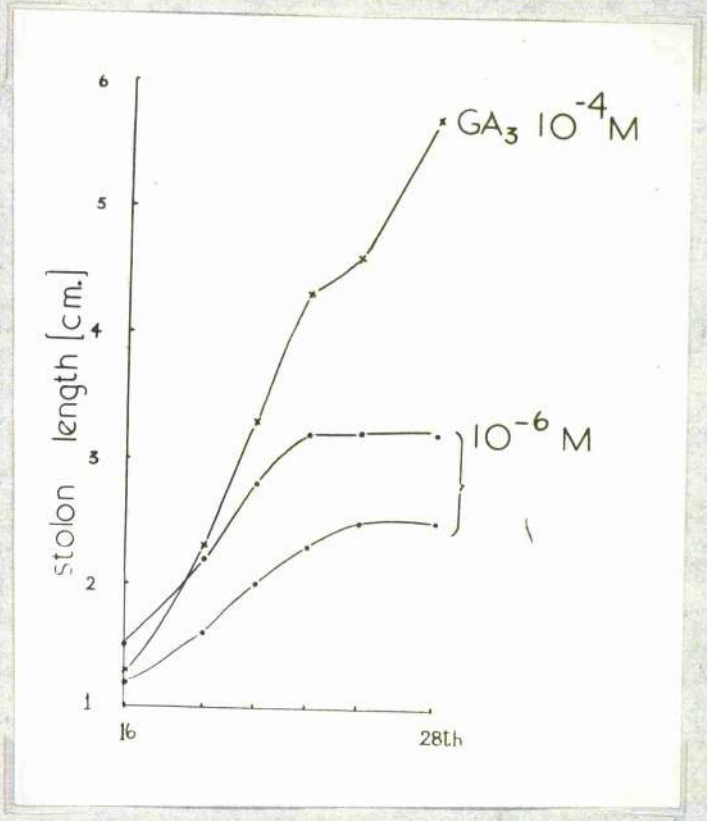


FIG. 30. Stolon growth in rosettes of L. uniflora treated with gibberellic acid for 24 hr. in dark. (Points on graphs are values for single stolons)

STOLON LENGTH [cm.] (6 plants per treatment)

DATE	CONTROLS	GA3 10^{-6}M	ETHREL 5mg/l	GA3 + ETHREL
8th July	1.0	0	0	4.1	1.6
10th	1.1	2.7	1.0	4.1	2.0
11th	1.2	3.0	1.4	4.1	2.2
15th	1.2	3.4	3.5	4.1	2.2

i.e. Only in GA-treated plants were stolons initiated under the experimental conditions. (In two rosettes out of six).

TABLE 28. Stolon initiation and elongation (Experiment 17)

RESULTS: Reference to table 26 (page 154) indicates that the responses to the three hormones is very variable, and may be so for the following reasons:

1. Matching of the rosettes may not have been accurate enough, which would result in different amounts of hormone being taken up by different rosettes in the same bathing solution. Endogenous hormone levels available for stolon development would also be different.
2. The 'initial' lengths of the stolons was not the same in each case, and so different parts of the growth rate curves are used as starting points. Furthermore the greater the differentiation of the terminal plantlet, the greater will be its control, if any, over any further elongation of the stolon.

Bearing the above points in mind the results were as follows:

ETHYLENE. Ethylene appeared to inhibit the elongation of the stolons and in fact brought about their senescence. Many of the initial lengths of the stolons were too great however to study this phase of growth definitively. With regard to morphological effects of ethylene, there was a promotion of the growth of the radial walls of the penultimate peripheral cells. Stolons contained an increased amount of starch grains compared to any of the other treatments.

GIBBERELLIC ACID: Figure 30 (page 156) indicates that there is a relationship between elongation and the concentration of exogenously supplied GA3. Both the rate of elongation (see table 27 (page 155)) and the final stolon length were increased by increasing the concentration of gibberellic acid, the final ratio of the lengths of the stolons being about 2:1 (6.7cm., 2.5cm.). These stolons were initially of similar length. By the 28th, the terminal plantlet on the above stolons had still not been fully differentiated. Thus gibberellic acid may bring about a protraction of the elongation phase of development of the stolon (at the expence of a delay in the differentiation of the offset). Gibberellic acid did not appear to promote elongation of the stolons if the initial lengths were more than 1.5cm.. Sectioned stolons showed a marked reduction in starch grains present compared with control turions, and the grains that were present were mainly limited to a central ring of cells.

IAA: In contrast to the above findings for gibberellic acid IAA promoted elongation of stolons, even if applied to stolons which were initially quite long (2.3cm.- 2.7cm.). The final lengths of the stolons (compared with controls (see table 27) (page 155)

of stolons were also increased in response to IAA treatment. Sectioned stolons had starch grains mainly localised in a central starch sheath, whilst in the controls starch grains had a more diffuse distribution. Stimulation of elongation may thus have been achieved through a production of soluble carbohydrates from starch reserves. The basal part of rosettes of this species when sectioned shows considerable starch reserves, and these are probably mobilised when required for the development of the primordia.

DISCUSSION: Table 28 (page 157) comprises some data from experiment 17 and is included in this section.

The effects of applied ethylene and IAA on stolon growth were so radically different, that an "IAA-induction of ethylene" hypothesis for IAA promotion of growth may be ruled out. Ethylene in the stolons under natural conditions may increase with increasing differentiation of the offset at presumably high IAA levels, and so bring about the cessation of growth observed at this point. Although the conclusion is very tentative because of the lack of replication, table 26 (page 154) indicates that such an inhibitory effect of ethylene would be particularly expressed when the gibberellic acid levels were low, as simultaneous application of gibberellic acid and ETHREL allowed some elongation. Since IAA could promote stolon elongation even if applied when the stolons were already about half their maximal length already, suggests that IAA may play a key role in this elongation phase of growth. Gibberellic acid in contrast was found to promote growth only if the stolons were initially short, which suggests that it may be involved in the early phases of stolon develop-

ment i.e. in their initiation. In experiment 17 only in plants treated with gibberellic acid was there any initiation of stolons under the experimental conditions. Cutter (1963) found with Hydrocharis morsus-ranae that application of either gibberellic acid or kinetin to the nutrient solutions of the cultures caused a four-fold increase in the number of stolons after 40 days, whilst if both were included then there was a 10-fold increase. It was suggested earlier that gibberellic acid (in results section) may be involved in the protraction of the elongation phase of growth of the stolons by delaying stolon senescence which is itself induced by the offset. Dusek et al (1965) reported for Azolla mexicana, where dispersal is a consequence of senescence of connecting stems between the rosettes, that gibberellic acid inhibited fragmentation by delaying senescence, whilst 10ppm. IAA increased the rate of fragmentation.

From the above observations it appears that gibberellic acid and IAA are both involved in the growth of the stolons, with perhaps the initiation of the stolons being controlled more by gibberellic acid and kinetin levels. If ethylene does have a role in stolon growth under natural conditions, then it may be as an agent of senescence and its effect will be particularly expressed if gibberellic acid levels are low.

CHAPTER 7.

NUTRITIONAL AND HORMONAL CONTROL OF LEAF GROWTH OF LITTORELLA UNIFLORA

EXPERIMENT 15.

AIM: This experiment was carried out to determine whether leaves of L. uniflora rosettes elongate at different rates on different muds, in the same depth of water. The experiment was presented as raw data for my honours exercise (1971).

METHOD: Plants and mud were collected from the Loch of Lowes, nr. Dunkeld in Perthshire, in shallow water on the N. shore, and also from the east shore of Lindores loch. Nine rosettes were grown per bin. Each bin contained 5kg. dry weight mud. Rosettes were visually matched. The rosettes were grown under 20cm. water in each bin.

RESULTS & DISCUSSION: Results are presented in table 29, page 164 page 165 and figure 31. The significances of the means differences are presented in table 30. p.164 Plate 40 illustrates representative rosettes from each treatment.

Lindores rosettes elongated more on Lindores mud than on Lowes mud, but elongated much more when grown on compost. Lowes rosettes likewise elongated more on Lindores mud than on Lowes mud, but did not respond to the same extent to the compost. The response of rosettes from Lindores and Lowes to Lindores mud was not significantly different (see table 30 1st 'block'). Lindores rosettes did elongate more than Lowes rosettes on Lowes mud ($P < 0.02$). Final leaf lengths of rosettes from Lindores and Lowes when grown on compost were in the ratio of about 2:1.

Thus the nutritional regime of a loch may dramatically promote or limit the elongation of the leaves of this rosette

species, even when the rosettes are in shallow water. The increase in leaf length first reported by Arber (1920) in response to depth of water may in some cases (assuming some nutrient uptake by the roots from the mud) actually be a response to changes in the light and nutritional regimes.

LINDORES ROSETTES	LONGEST LEAF PER ROSETTE (MEANS ± S.E.) (cm)	
LINDORES MUD	6.57 ± 0.43	(9)**
LOWES MUD	5.20 ± 0.33	(9)
COMPOST	12.05 ± 0.30	(11)
LOWES ROSETTES		
LOWES MUD	3.93 ± 0.35	(9)
LINDORES MUD	6.10 ± 0.29	(9)
COMPOST	6.18 ± 0.64	(10)

** No. in brackets indicates no. replicate rosettes.

TABLE 29. Leaf lengths of rosettes of L. uniflora grown on three muds.

	SIGNIFICANCE LEVELS OF MEANS DIFFERENCES ('t' test)	Signi-ficance
LINDORES ROSETTES/LOWES ROSETTES...	a. LINDORES MUD	(NS)
	b. LOWES MUD	(P 0.0
	c. COMPOST	(P 0.001
LINDORES ROSETTES ...	LINDORES MUD/LOWES MUD	(P 0.05)
	LINDORES MUD/COMPOST	(P 0.001
	LOWES MUD/COMPOST	(P 0.001
LOWES ROSETTES.....	LOWES MUD/LINDORES MUD	(P 0.001
	LOWES MUD/COMPOST	(P 0.01)
	LINDORES MUD/COMPOST	(NS)

TABLE 30. Significance of means differences from table 29.

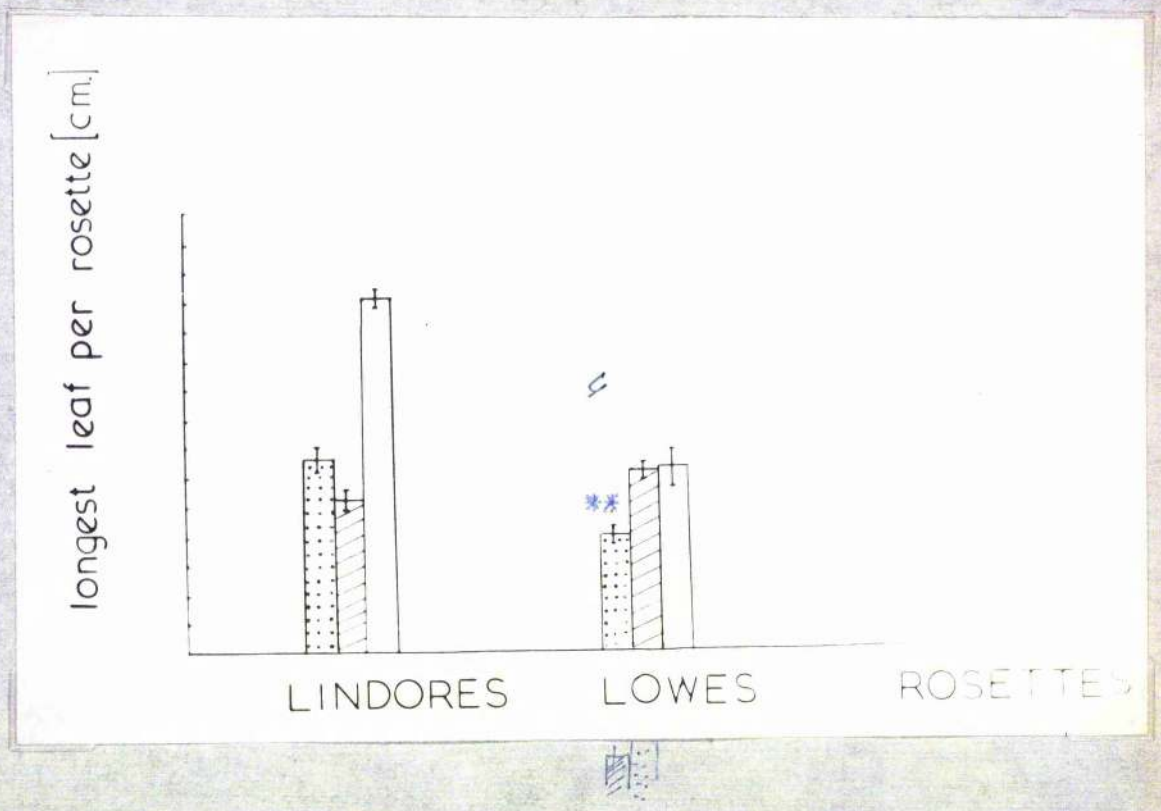


FIG. 31. Leaf growth of rosettes of L. uniflora, from Lindores loch, and the Loch of the Lowes, grown on 3 muds.

LEGEND: Stippled: Lindores mud

Striped: Lowes mud.

Blank: Compost.

** Errata: 1st histogram should be striped, and 2nd stippled with depicted values.

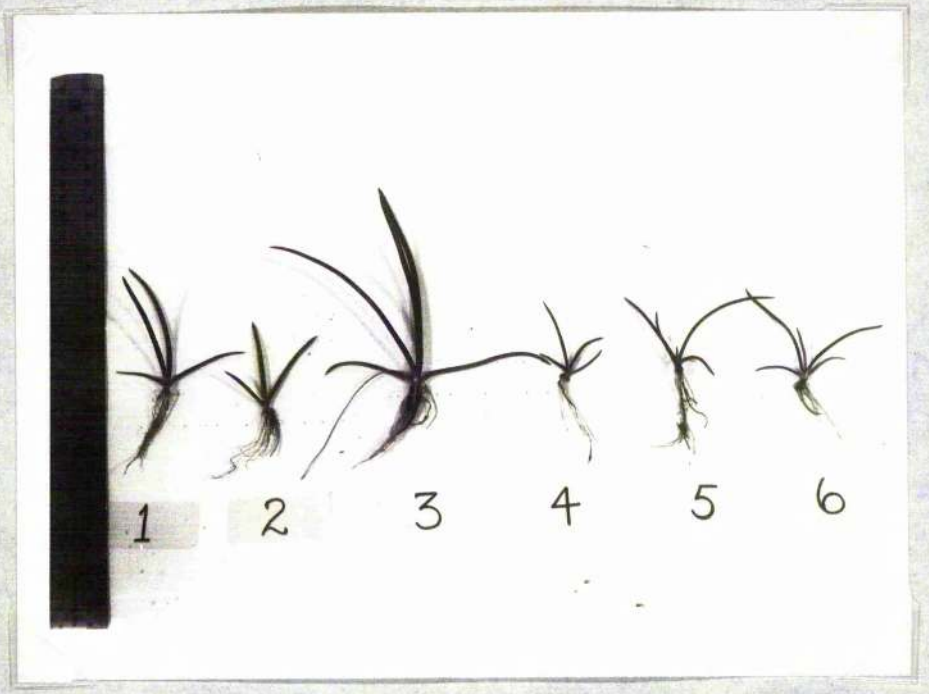


PLATE 40. Response of rosettes to different muds.

1-3 LINDORES ROSETTES grown on Lindores mud, Lowes mud, compost.

4-6 LOWES ROSETTES grown on Lowes mud, Lindores mud, compost.

EXPERIMENT 16.

AIM: To determine if ethylene supplied in the form of ETHREL E can cause elongation of the leaves of Littorella uniflora L. Aschers.

MATERIALS: Rosettes were collected from Lindores Loch on 31st May, 1974. ETHREL E was obtained from Mark's & Co. Ltd.. The nutrient solution used was Hoagland's half-strength.

METHOD: Six rosettes were held in the aerial, floating and submerged positions in 250ml. half-strength Hoagland's solution with or without 0.5mg/litre ETHREL, in Petri dishes with two replicate dishes per treatment. Plastic covered wire-netting was used to hold the rosettes in each position. The total leaf length of each rosette was measured before and after the experimental period of 10 days. Loss of chlorophyll was noted in some leaves by this time. The rosettes were held under daylight at 20°C.

RESULTS: No increase in total leaf length less than 0.5cm. was considered to be of experimental significance or representative of growth. The summarised results are presented below in table 31.

Change in Total Leaf Length per Rosette.(cm.)

AERIAL		FLOATING		SUBMERGED	
-E	+E	-E	+E	-E	+E
7.7-8.2	10.6-12.3	8.5-9.0	7.2-8.0	8.3-9.9	6.3-8.5
		8.9-9.9	9.5-10.2		7.1-9.9
		9.7-10.4	9.6-10.1		8.2-9.7
		9.8-10.7			9.0-9.5
					9.0-9.7
					9.2-10.1
					9.9-11.0
					10.3-11.5
					11.8-13.5
(11X)	(11X)	(8X)	(9X)	(11X)	(3X)

'X' indicates the number of rosettes that did not show any significant growth in each treatment.

TABLE 31. Leaf growth of L. uniflora in the aerial, floating and submerged positions, with and without 0.5mg/litre ETHREL E.

DISCUSSION: In contrast to the findings of Musgrave et al (1972) with Callitriche platycarpa where supplied ETHREL could not promote the elongation of stems of submerged rosettes, the leaves of Littorella uniflora rosettes were able to respond to supplied ETHREL when submerged (see table 31) suggesting that under shallow water conditions ethylene accumulation or synthesis under natural conditions may be low. Unlike Callitriche platycarpa which grows aerially or just submerged, rosettes of Littorella uniflora are able to grow over a considerable depth range, where there must be changes in hormone levels or changes in the sensitivity of the tissues to hormone levels, induced and effected by variation in the light regime, and carbon dioxide concentration. With many aquatic plants what must be remembered is that they are subjected to submergence by different depths of water. The response of the rosette, when submerged, to ethylene and gibberellic acid is considered in the following experiment.

EXPERIMENT 17.

AIM: To study the effects of supplied gibberellic acid and ethylene on the elongation of the rosettes of Littorella uniflora.

MATERIALS: Rosettes were collected from Lindores Loch. The following bathing solutions were used:

10^{-6}M GA3	ETHREL 5mg/litre.
-	-
+	-
-	+
+	+

METHOD: Six rosettes were held in 1000ml. of each of the above bathing solutions for 24hr. in the dark at 20°C. The rosettes were removed from the bathing solutions, washed in distilled water, scored individually for total leaf length and then potted. The rosettes were held under 25cm. water in daylight. The experiment was set up on the 8th July, 1974 and the rosettes were remeasured 7 days later, when new growth was evident.

RESULTS: The results are presented in tables 32 and 33, p. 171 Over the experimental period gibberellic acid had no effect on the elongation of the leaves. Treatment of rosettes with ETHREL alone inhibited elongation of the rosettes, whilst simultaneous supply of gibberellic acid with the ETHREL nullified this inhibitory effect. The inhibitory effect of the ETHREL may have been a concentration effect for in the previous experiment it was shown that 0.5mg/litre ETHREL promoted the elongation of the rosettes. This experiment indicates that where gibberellin levels are low in the rosettes then growth will be very slow in the presence of

accumulated ethylene. Stolons developing under the experimental conditions were measured and the results are included in chapter 6 concerned with stolon growth.

In the following experiment the effect on leaf elongation of a higher concentration of gibberellic acid (10^{-4}M/litre) is studied. The response of the rosettes to supplied IAA is also studied, as high IAA concentrations are known to stimulate ethylene formation in some tissues, and thus a comparison with the above results will be possible.

INITIAL RANGE IN TOTAL LEAF LENGTH	CONTROLS	GA3	ETHREL	GA3+ETHREL
6-8cm.	6.0-7.4	--	6.9-7.1	6.1-7.6
	7.0-8.2		7.0-8.6	
8-11cm.	9.1-9.8	8.0-10.2	9.7-10.2	8.0-10.4
	9.2-10.7	8.7-9.8	10.3-10.3	8.8-8.8
	9.7-10.4	10.7-12.4	10.5-11.0	9.1-10.3
	10.6-12.3			9.4-11.3
11-14cm.	--	14.0-14.0	12.5-13.0	11.8-13.2
		14.1-15.0		

TABLE 32. Leaf growth in rosettes treated with ethrel and gibberellic acid.

INCREASE IN TOTAL LEAF LENGTH (initially 8-11cm.) OF ROSETTES.

CONTROLS	GA3	ETHREL	GA3 + ETHREL
0.7	2.2	0.5	2.4
1.5	1.1	0.0	0.0
0.7	1.7	0.5	1.2
1.7			1.9
$\bar{x}$ 1.15	1.67	0.33	1.83
SD. 0.45	0.45	0.24	0.49

't' test on means for different treatments indicates that only ETHREL had an effect on growth ($P<0.05$) and that this was an inhibitory effect on elongation.

TABLE 33. Leaf elongation in response to ethylene and gibberellic acid.

EXPERIMENT 18

AIM: To investigate possible hormonal factors controlling the induction and elongation of the leaves of Littorella uniflora L. Aschers.

MATERIALS: Rosettes were collected from Loch Lindores (26th August, 1974) and were selected for the experiment if they had four similar leaves, and had a total leaf length of 11-12cm. Table 34 presents the initial variation in the rosettes used.

TREATMENT	INITIAL TOTAL LEAF LENGTHS PER ROSETTE (cm.) (MEAN ± S.E.)
CONTROL	11.6 ± 0.23
GA3 10^{-4}M	11.7 ± 0.23
IAA 10^{-6}M	11.2 ± 0.22
IAA 10^{-4}M	11.0 ± 0
IAA 10^{-3}M	11.7 ± 0.23

TABLE 34. Initial total leaf length per rosette.

Loch mud used in the experiment was collected from beneath the sward.

METHODS: The rosettes (4 per treatment) were exposed to the above bathing solutions (1 litre) for 24hr. in the dark, and then washed with distilled water. They were then potted singly in small containers filled with loch mud, and held below 20cm. water at 20°C. The rosettes were illuminated by 3 gro-lux tubes (3 foot, 40 watt each) for 14hr. per day. The three gro-lux tubes were held 20cm. above the water in the glass tanks in which four replicate rosettes per treatment were held. The duration of the experiment was from 26.8.74 - 18.9.74, at the end of which

the rosettes were scored for various growth parameters. Differences between treatments were visible by this date.

RESULTS: Results are tabulated in tables 35-39.

TREATMENT	LENGTHS OF LEAVES (cm.)	NEW LEAF TISSUE PER ROSETTE (cm.) (MEANS±S.E.)	NO. NEW LEAVES
CONTROL	1.0/2.3,0.9/1.3/2.4	1.97±0.45	1.25±0.43
GA3 10^{-4}M	5.1/5.9/4.1/5.4,4.8	6.32±1.15	1.25±0.43
IAA 10^{-6}M	1.5/2.5,2.0,1.1/1.7,0.5,0.5/1.1	2.72±0.85	2.00±1.00
IAA 10^{-4}M	1.5,0.5/1.4,0.5/1.1,1.4/2.2,1.2	2.45±0.30	2.00±0
IAA 10^{-3}M	0.8/2.0/0.6/1.1	1.12±0.25	1.00±0

TABLE 35. Formation and elongation of leaves. Figures between each oblique are values for individual rosettes.

a. NEW LEAF TISSUE PER ROSETTE

CONTROL		CONTROL	(NS)	CONTROL	(NS) CONTROL
GA3 10^{-4}M	P 0.01	IAA 10^{-6}M		IAA 10^{-4}M	IAA 10^{-3}M

b. LEAF INITIATION No treatment had a significant effect on leaf initiation.

TABLE 36. Significance levels of means difference.

TREATMENT	NO. SENESCENT LEAVES	MEAN ± S.E.
CONTROL	-/1/-/1	0.5 ± 0.25
GA3 10^{-4}M	-/2/-/-	0.5 ± 0.45
IAA 10^{-6}M	1/1/1/-	0.75 ± 0.20
IAA 10^{-4}M	1/-/1/-	0.5 ± 0.25
IAA 10^{-3}M	2/3/4/2	2.75 ± 0.4

Compared to control, only IAA 10^{-3}M had an effect on leaf senescence ($P < 0.01$)

TABLE 37. Leaf senescence.
Figures between obliques are values for individual rosettes.

TREATMENT	ROOT LENGTH (cm.) MEAN ± S.E.
CONTROL	17.3 ± 2.0
GA3 10^{-4}M	17.0 ± 3.7
IAA 10^{-6}M	19.2 ± 1.3
IAA 10^{-4}M	13.1 ± 0.6
IAA 10^{-3}M	11.1 ± 1.5

't' test indicates that no treatment had a significant effect on total root length per rosette.

TABLE 38. Total main root lengths. (cm.)

TREATMENT	ROOT LENGTH (cm.)	NO. LATERALS	ROOT LENGTH	NO. LATERALS	ROOT LENGTH	NO. LATERALS
CONTROL	2.0	-	1.3	-	1.7	-
	4.7	-	2.0	-	1.8	-
	5.8	2	5.0	3	5.0	-
			5.7	3	5.0	2
			6.3	5	5.0	2
GA3	1.5	-	3.0	-	2.5	-
	1.5	-	4.0	-	5.0	-
	1.8	-	5.0	-	6.0	-
	2.0	-			6.0	10
	6.2	15			6.5	-
IAA 10^{-6}M	1.5	-	2.2	-	1.0	-
	2.5	-	2.5	-	5.4	-
	4.0	-	3.2	-	6.0	-
	4.1	-	4.5	-	6.0	3
	5.0	-	4.5	2		
	5.1	-				
IAA 10^{-4}M	2.0	-	1.6	-	2.0	-
	2.5	-	3.0	-	4.0	-
	3.0	-	4.2	8	5.5	2
	6.5	13	5.0	8		
IAA 10^{-3}M	2.0		0.5		1.0	
	3.0		3.0		1.0	
	3.1	NONE	3.2	NONE	2.0	NONE
			5.6		2.0	
			4.2		2.3	
					2.5	

TABLE 39. Lateral root production. (Figures for three rosettes per treatment.)

RESULTS AND DISCUSSION:

Leaf initiation. Neither gibberellic acid nor IAA affected the initiation of leaves during the experimental period (see tables 35,36 page 173. Fewer leaves were initiated in rosettes from the 10^{-3}M IAA treatment. This may have been due to the promotion of the senescence of the originally metabolically active leaves, which would have been supplying hormones and other growth factors to the meristem. Cytokinins may therefore play a more important role than IAA or gibberellic acid in leaf initiation, as they also are known in some species to be involved in leaf growth.

Leaf elongation. In contrast to its having no effect on leaf initiation gibberellic acid caused a marked increase in the elongation of leaves formed during the experimental period. IAA had no significant effect on the elongation of newly formed leaves. (see table 35).

The following observations were made concerning growth of leaves treated with gibberellic acid. There was no elongation of the original leaves of the rosettes in response to gibberellic acid, i.e. mature leaves had lost their capacity to respond to higher hormone levels (suggesting perhaps the formation and localisation of growth inhibitors in mature leaves of the rosettes). Leaves elongating in response to gibberellic acid grew more erect than do the leaves of rosettes normally growing in shallow water, where there is greater growth of the upper halves of the leaves resulting in leaf curvature.(see plate 36) page 147 Furthermore, this elongation phase in response to gibberellic acid seemed to be followed by a phase of radial growth. Leaf diameters were measured in each treatment, but no treatment had any significant

effect. This may however follow simply because the diameters were only measured once (not tabulated) for the leaves elongating in response to gibberellic acid were initially of small diameter. The erect nature of the growth of the leaves in response to gibberellic acid suggests that the increase in the elongation of rosettes with depth may be due to increased levels of gibberellic acid. Where light intensities are low, or 'etiolating', plant growth in general assumes a more vertical nature. The radial growth of the leaves will be controlled by the factors controlling lacunal development. Since ethylene is probably one of the internal gases, it may be responsible for lateral rather than longitudinal growth of the rosettes, under conditions where gibberellins are low.

Leaf senescence. The only treatment to affect leaf senescence was 10^{-3}M IAA, which promoted the senescence of the mature leaves of the rosettes (see table 37)(page 174). Young leaves in these rosettes were still healthy at the end of the experimental period, suggesting an ethylene effect. In many terrestrial plants it is known that abscission and senescence of leaves is promoted by ethylene when IAA levels are low. Presumably the younger leaves of the rosettes had higher levels of hormones than did the older leaves. Ethylene synthesised in growing regions of the rosettes may be transported via the extensive lacunar system to the mature leaves and so effect their senescence. The senescence of the leaves is marked by the loss of chlorophyll, and a slight reddening of the leaves. Such effects of ethylene on fruit maturation are well documented. Alternatively this effect of IAA may simply have been through infiltration of the lacunar system with

water as a consequence of tissue damage.

Root growth. Table 38, (page 174) presents the final root lengths. No treatment apparently had any effect on total length of main root. This may appear so partly because of initial variation in roots. It was observed that in 10^{-4}M and 10^{-3}M IAA-treated rosettes, the roots were much thicker than the controls, i.e. there was some promotion of radial growth of the roots. High concentrations of both IAA and kinetin were reported by Svensson (1972) in wheat and maize to bring about radial growth of roots at the expense of longitudinal growth. Gibberellid acid-treated rosettes produced thinner roots. Svensson likewise found this to be the case with wheat and maize. None of the effects reported by Svensson were due to induced ethylene, for applied ethylene had no effect on root growth. He did however report both kinetin and gibberellin stimulation of ethylene synthesis in the roots.

Lateral root production. In all treatments lateral roots only occurred on main roots greater than 4cm. in length (see table 39) (page 175). This may be partly due to the reported inhibitory influence of the root tip. Thimann (1936) first reported the inhibitory effect of the root tip on lateral root development. This influence is not solely an auxin effect for the inhibitory influence could not be reasserted by replacement of the root tip with a supply of auxin. Torrey (1959) isolated a phenolic inhibitor from the root tip, which he believes was the inhibitory influence. Little really is known concerning lateral root formation. They do arise normally adjacent to protoxylem poles. Growth in a closed system has been reported by Talbot & Street (1958a) to stimulate

lateral root formation in wheat. They did not eliminate the possibility of the involvement of ethylene, because of the insensitivity of their measuring. Both gibberellic acid and 10^{-4}M IAA promoted the formation of lateral roots on main roots of a similar size to those in the control rosettes. Lack of supply of these hormones from the leaves, in the control rosettes, may account for the low production of laterals, and this may be affected by the light regime. Furuya & Torrey (1964) found that red light inhibited lateral root formation, whilst far-red light reversed this inhibition. From their findings they postulated that under natural conditions perhaps a phytochrome-mediated product translocated from shoots to roots, might be involved in lateral root formation. Since lateral root formation is a process, rather than an isolated event, involving dedifferentiation, determination, and development of the root primordia, there is likely to be considerable hormonal interaction, with different stages requiring different hormone concentrations.

Summary.

1. Neither gibberellic acid nor IAA affected leaf initiation suggesting that perhaps cytokinins are more closely involved with this process.

2. Gibberellic acid stimulated leaf elongation but only of leaves initiated during the experimental period, i.e. it had no effect on mature leaves. IAA had no effect on leaf elongation. Comparison with the situation in the field indicates that perhaps gibberellic acid levels are greater in deeper water plants.

3. Only high IAA affected leaf senescence. This may have

been an ethylene effect or simply due to tissue damage.

4. No treatment affected the total root length. There was some promotion of radial growth of roots with rosettes exposed to 10^{-4}M and 10^{-3}M IAA. Roots produced in rosettes exposed to gibberellic acid were thinner.

5. Both gibberellic acid and high IAA promoted lateral root formation, suggesting that they are normally transported from the leaves to the roots.

SUMMARY.

LITTORELLA UNIFLORA (L.) ASCHERS

1. The life cycle of this rosette aquatic plant was discussed in detail.

2. Anatomical differences between terrestrial and submerged forms of the rosettes were found. Terrestrial rosettes were induced very rapidly by exposure of previously submerged rosettes to air. Lacunal development was greatly reduced in the leaves and roots of the terrestrial rosettes.

3. Two main aspects of the life cycle were considered in detail: propagation of the rosettes; and leaf elongation.

4. Propagation: In nature the rosettes have the ability to propagate by (a) stolon formation or (b) by a less efficient means where the rosette elongates vertically.

(a) Experimental evidence presented indicates that stolon initiation is controlled by gibberellic acid, although cytokinins may be involved. In nature, stolons from similar rosettes may be of varying lengths, and so the elongation phase of stolon development was considered. It was found that gibberellic acid may bring about a protraction of this elongation phase of growth of the stolon, at the expense of the development of the terminal plantlet. Ethylene supplied as ETHREL E inhibited further elongation of the stolons, and furthermore, promoted accumulation of starch. Starch grains in stolons of rosettes treated with gibberellic acid were limited to a central ring of cells. Some radial growth in response to supplied ETHREL E was noted. Stolons may persist in

some cases, thus interconnecting many rosettes, but in some cases the stolons senesce. Evidence presented above suggests that gibberellic acid and ethylene may control senescence. Where gibberellin levels are low in the stolons, accumulated ethylene will rapidly effect the senescence of the stolon, thus making the rosette independent.

(b) Where rosettes are partially and repeatedly buried by sediment, they have the ability to perennate themselves vertically by means of an elongated stem axis. Various rooting levels on the same rosette reflect the sequential nature of the deposition of the sediment. This was found experimentally where rosettes were grown under different conditions of deposition of sediment, and rosettes similar to those experimentally induced were found on a silt fan, near the inflow in Loch Drumore, near Glenshee, in Perthshire. The ability of the plant to propagate so rapidly by stolon formation on eroded shores, or by altering its rooting level and then forming stolons, explains to a great extent the ubiquity of this species throughout Great Britain, and thus implicitly explains the persistence of this species through many of the stages in hydrosere formation.

5. Leaf elongation: Leaf elongation of the rosettes is known to increase with the depth of water in which the rosettes are growing. What is not known is whether this is a function of a changing mud, with an increase in the silt fraction with depth, or whether it is due to the changing light regime, or to both factors.

Experimental evidence presented demonstrates that even

in shallow water the leaf elongation of the rosettes may be markedly controlled by the nature of the mud. Thus leaf elongation is partially under nutritional control, and explains the increase in leaf elongation seen in nature, with increasing depth of water. Where there is little change in mud however with depth, the control of leaf elongation must lie elsewhere. The main other variant will be in the light regime.

Factors affected by the light regime will be the levels of hormones such as gibberellic acid, ethylene and auxin. Leaves of the rosettes were able to respond to supplied ETHREL B when submerged, thus indicating that in shallow water ethylene production may be low in the rosettes. In the saturated system described for Callitriche platycarpa by Musgrave et al (1972) the stems of the rosettes were only able to respond to supplied ETHREL when floating. It is suggested that ethylene may be more actively involved in the radial growth of the leaves, for its stimulation of leaf elongation was not great. Gibberellic acid however, when supplied exogenously stimulated the elongation of leaves initiated during the experimental period. IAA had no effect on leaf elongation, and at levels where induction of ethylene would be expected (10^{-4}M) still no stimulation of elongation occurred. It is suggested that anthocyanin formation in the leaves of the rosettes, lacunal development and hence radial growth are under the control of ethylene, whilst gibberellins have more control over leaf elongation. Such a postulate implies an increase in gibberellins or in turnover of gibberellins with increase in the depth of water, a similar theory to that first forwarded by McComb (1965)

but later discounted by Musgrave et al (1972). Both workers were however considering Callitriche species which are either floating or submerged, whilst the rosettes of Littorella uniflora are subjected to degrees of submergence, some of which may affect the light regime significantly to alter hormone levels. Aberg (1943) presented for Lobelia dortmanna, a similar rosette species, field data that indicated that increasing light attenuation brought about increasing elongation of the leaves.

5. High auxin levels in the bathing solutions promoted radial growth of the roots whilst 10^{-4}M gibberellic acid reduced root diameter. Lateral root formation was promoted by high auxin concentrations and also by gibberellins. There is considerable promotion of lateral roots on aerial exposure of rosettes, and this may be brought about by changes in the levels of these compounds within the rosettes.

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Alastair C. Webster.

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